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PROCEEDINGS OF
THE SECOND DEFOLIATION CONFERENCE,
5 - 6 AUGUST 1964

compiled by

Robert A. Darrow
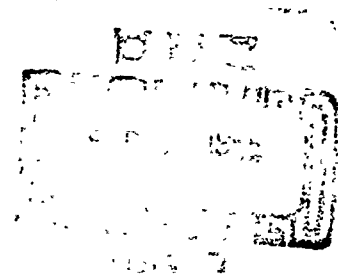Vesta Z. Mattie

AUGUST 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

SECURITY

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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

PROCEEDINGS OF THE SECOND DEFOLIATION CONFERENCE,
5-6 August 1964

compiled by

Robert A. Darrow
Vesta Z. Mattie

Crops Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1C522301A06101   August 1965

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FOREWORD

This Proceedings of the Second Defoliation Conference of the U.S. Army Biological Laboratories presents a record of research progress in our knowledge of the process and mechanisms of natural defoliation or leaf abscission and in the search for effective chemicals that induce defoliation.

New information has been developed in both contract and in-house research programs concerning the role of ethylene and other endogenous substances that stimulate leaf abscission. In vitro production of ethylene from cell-free plant extracts has been measured for the first time in biochemical studies of ethylene synthesis in plants. Detailed studies have been made of the structural changes that take place in the abscission layers of leaves during the abscission process.

Contract synthesis and screening programs have been expanded to include a large number of contract firms in the search for effective defoliants. This concentration of effort on synthesis has led to the discovery of several new groups of biologically active compounds. A number of chemicals have been investigated that show defoliant or desiccant activity in primary screening programs at rates of 0.1 pound per acre.

In-house screening and field test activities have been expanded. Several of the newly synthesized candidate agents have been included in field test programs.

ABSTRACT

The research program in chemical defoliation of the U.S. Army Biological Laboratories and its affiliated contractors and agencies is summarized in this Proceedings of the 1964 Defoliation Conference.

Four phases of the effort are reviewed: (i) basic studies in the mechanisms of leaf abscission by contract and in-house research; (ii) synthesis of new chemicals as candidate defoliants, desiccants, or herbicides; (iii) screening and evaluation of candidate chemicals by in-house and contract programs; and (iv) field testing of promising defoliants under in-house and USDA programs.

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AGENDUM

SECOND DEFOLIATION CONFERENCE
5-6 August 1964

Chairman: Dr. Robert A. Darrow

5 August 1964

0900-0910	Announcements & Introductions	
0910-0920	Welcome	Colonel Vincent L. Ruwet Commanding Officer
0920-0935	Defoliation in Counterinsurgency Operations	Brig. Gen. Fred J. Delmore CG, USA Edgewood Arsenal
0935-0950	U.S. Army BioLabs Defoliation Program	Dr. Robert J. Goodlow Director of Biological Research
0950-1005	Coffee Break	
1005-1050	In-House Basic Research on Leaf Abscission	Lt. Bernard Rubinstein Dr. Fred B. Abeles
1050-1140	University of California Contract Program	Dr. F.T. Addicott
1140-1230	Purdue Contract Program on Leaf Abscission	Dr. D.J. Morre Mr. Peter Scott
1230-1330	Lunch	

SYNTHESIS PROGRAMS

1330-1350	Pennsalt Chemicals Corporation	Dr. Herbert Q. Smith
1350-1410	Ethyl Corporation	Dr. John Wollensak
1410-1430	Monsanto Research Corporation	Dr. Stanley D. Koch
1430-1450	General Aniline & Film Corporation	Dr. E.O. Leonard
1450-1500	Coffee Break	

1500-1520	Coding and Data Processing of Chemical Information	Mr. William J. Wiswesser
1520-1540	Dow Chemical Company	Dr. J.K. Leasure
1540-1600	FMC Niagara Chemical Division	Dr. Joe R. Willard
1600-1620	Hooker Chemical Corporation	Dr. Edward D. Weil
1620-1640	American Cyanamid Company	Dr. R.J. Magee
1640-1700	General Discussion	
1800-1900	Cocktail Hour	
1900-	Dinner at Holiday Inn Speakers: Captain Carl W. Marshall, USAF Captain Charles Hagerty, USAF	

SCREENING PROGRAMS

6 August 1964

0830-0910	Crops Division Defoliation Screening	Dr. R.A. Darrow Mr. J. Ray Frank
0910-0940	Pennsalt Chemicals Corporation	Dr. Herbert Q. Smith
0940-1010	Ethyl Corporation	Dr. John C. Wollensak
1010-1020	Coffee Break	
1020-1050	Monsanto Research Corporation	Dr. Philip C. Hamm
1050-1120	Dow Chemical Company	Dr. Keith Barrons
1120-1150	U.S. Department of Agriculture ARPA Program	Dr. Dayton L. Klingman
1200-1315	Lunch at the Officers Open Mess	
1315-1345	Crops Division Field Test Program	Mr. Kenneth Demaree
1345-1430	General Discussion	
1430-	ETD	

I. RELATIONSHIP BETWEEN LEAF ABSCISSION AND ETHYLENE EVOLUTION

Bernard Rubinstein* and Fred B. Abeles**

Our efforts during the past year have been directed primarily toward learning the mechanism of leaf abscission and, more specifically, to investigating the nature of abscission stimulations. Drawing on previous work by Hall and co-workers,[1-3]*** we have attempted to correlate the presence of internally produced ethylene with the onset of bean leaf abscission.

Phaseolus vulgaris L. var. Black Valentine was used as the test plant. Abscission measurements involved the use of one-centimeter sections of primary leaf petioles with the abscission zone centrally located. These explants were sealed in bottles and the air was withdrawn at intervals and injected into a gas chromatograph.[4]

Abscission of bean petioles has been shown previously to take place in two stages.[5] Thus, immediate applications of naphthaleneacetic acid (NAA) inhibit abscission; later applications stimulate abscission regardless of concentration or site of application. The NAA causes marked increases in ethylene production during both stages, but the amount of ethylene present during stage 2 can be correlated with the acceleration of abscission.[4]

Chatterjee and Leopold[6] found that the ability of different phenoxyacetic acids to promote abscission during stage 2 was directly related to the growth activity of these compounds. To examine any correlation between ethylene evolution and induction of abscission, explants in stage 2 were transferred to agar containing various substituted phenoxyacetic acids and the ethylene evolved was measured 12 hours later.

Data are shown in Figure 1 with the compounds arranged according to increasing abscission activity from left to right. There appears to be an approximate correlation between stimulatory action on abscission and ethylene production, but the same concentrations (10^{-4}M) were used throughout this experiment. These tests are not directly comparable to those of Chatterjee and Leopold,[6] who used the minimum concentration that would inhibit abscission during stage 1. In general, however, compounds that are active growth regulators and hence potent abscission stimulators (2-5-dichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid) produce more ethylene than the less active compounds.

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To investigate any relationship between ethylene evolution and abscission, one must also consider the wide variety of nonauxinic compounds that have profound effects on leaf abscission. Rubinstein and Leopold[7] found that amino acids have varying degrees of activity on the bean petiole abscission test; alanine and glutamic acid are among the most stimulatory, and valine and leucine are relatively inactive. These amino acids were applied to explants in gas collection bottles and the ethylene evolved was measured over time (Fig. 2). The pattern of ethylene evolution is quite different from the immediate increases in gas production following applications of NAA.[4] No differences can be seen from the controls until 36 hours after the explants are cut. At that time ethylene evolution is markedly increased by the two amino acids that stimulate abscission. Abscission occurs 12 hours after the ethylene acceleration. The amino acids leucine and valine that are ineffectual promoters of abscission have little effect on ethylene evolution.

Amino acids were further analyzed as to their effects on the two abscission stages. The four amino acids were added to bean explants at intervals up to 12 hours after cutting to observe effects on stage 1 and until 48 hours after cutting to observe effects on stage 2. As shown in Table 1, the amino acids had no effect on either ethylene production or abscission rate when applied from 0 to 12 hours after cutting. If applied 48 hours after cutting, however, alanine and glutamic acid markedly increased both ethylene evolution and the rate of abscission. Leucine and valine still had no effect on rate of ethylene evolution, and these explants abscised at about the same times as the controls.

Similar analyses were also performed with some common defoliants. Endothal and two of its inactive analogs, methylene endothal and Δ-methylene endothal (Fig. 3) were applied to bean explants along with potassium iodide (KI) and the ethylene evolution was measured over time (Fig. 4). Both active defoliants, endothal and KI, caused increases in ethylene evolution—the endothal showed immediate stimulations with a subsequent decline and KI stimulated ethylene increasingly for 24 hours before tapering off. The analogs of endothal, methylene endothal and Δ-methylene endothal, which are relatively ineffective defoliants, showed only slight differences from the controls.

The effect of the various defoliants on the two stages of abscission is shown in Table 1. Both endothal and KI stimulated ethylene production during stage 1 (applications from 0 to 12 hours) but abscission was inhibited. When these compounds were applied during stage 2 (after 48 hours had elapsed), ethylene production was stimulated once again, but the abscission rate was also markedly accelerated. Methylene endothal and Δ-methylene endothal showed a slight stimulation of ethylene evolution during stage 1 but had no effect if applied during stage 2. Abscission rates of explants treated with these two endotahl analogs were not different from the control.
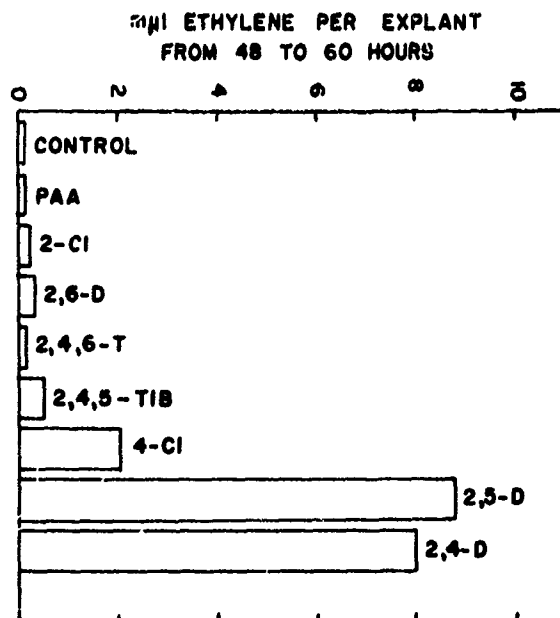
Figure 1. Effect of Various Phenoxyacetic Acids
on Ethylene Production of Bean Petiole
Abscission Zones. Explants were in
plain agar for 48 hours before being
transferred to test substances.
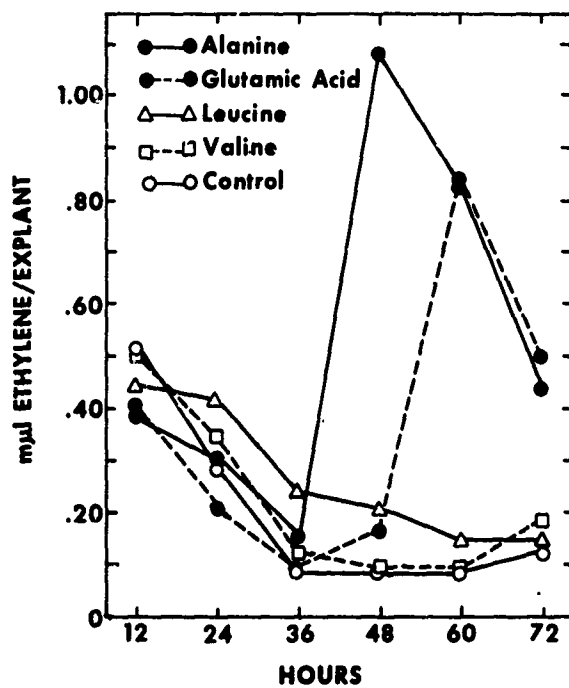
Figure 2. Effect of Amino Acids on
Ethylene Production by
Bean Petiole Explants.

TABLE 1. COMPOUNDS AFFECTING ETHYLENE PRODUCTION AND RATE OF ABSCISSION WHEN APPLIED TO BEAN EXPLANTS

| | Hours Elapsed Before Applying Compound: | | | |
| | 0 | | 48 | |
Compound	Ethylene Evolved 12 Hours Later, mμl/explant	Hours to 50% Abscission[a]	Ethylene Evolved 12 Hours Later, mμl/explant	Hours to 50% Abscission[a]
Amino Acid				
L-Alanine	0.57	74	0.75	59
L-Glutamic Acid	0.51	71	0.62	61
L-Leucine	0.54	70	0.10	74
L-Valine	0.51	74	0.08	73
Control	0.52	75	0.10	75
Defoliant				
Endothal	6.73	>100	8.51	58
Methylene Endothal	0.71	70	0.15	68
Δ-Methylene Endothal	0.75	73	0.13	73
Potassium Iodide	1.33	98	3.21	56
Control	0.55	75	0.10	75

a. Standard error was never greater than ± 4 hours.
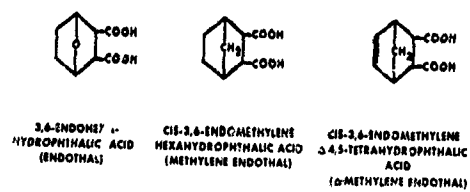
Figure 3. Molecular Structures of Endothal and Two of Its Inactive Analogs.
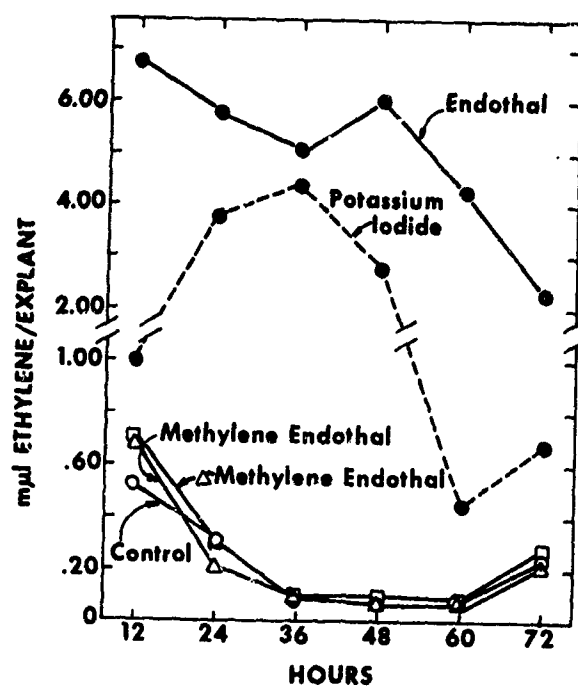
Figure 4. Effect of Defoliants on Ethylene Production by Bean Petiole Explants.

From data presented, we feel that endogenously produced ethylene may be
implicated in the abscission processes. Large numbers of abscission-
accelerating substances were applied to bean petiole explants and the
evolution of ethylene always preceded the stimulation of abscission. For
example, certain phenoxyacetic acid compounds that are active as growth
regulators are active promoters of abscission during stage 2.[6] These
compounds also markedly stimulated ethylene evolution. The amino acids
alanine and glutamic acid did not immediately stimulate ethylene evolution
but 12 hours before abscission could be observed, measurable amounts of
ethylene were produced. Explants treated with the latter two amino acids
48 hours after cutting evolved large amounts of ethylene immediately after
placement.

Both endothal and potassium iodide instantly stimulated ethylene evolu-
tion, but when these substances were applied immediately after the abscis-
sion zone explants were excised, no abscission occurred. If the same con-
centration of defoliants was applied when explants were in the second
stage (48 hours after cutting), stimulation of ethylene evolution was
again observed and at that time abscission occurred rapidly. These
results show for the first time that direct applications of defoliants
to the abscission zone can stimulate abscission. It is possible that
the defoliants injure the explants and retain them in the first stage.
Ethylene, as reported earlier,[4] is ineffective as an abscission stimulant
during stage 1.

Along with the findings that compounds that promote abscission likewise
increase ethylene evolution is the observation that other substances that
have little effect on explant abscission are unable to stimulate the pro-
duction of ethylene. Phenoxyacetic acids that are ineffectual growth
regulators also produced few changes in time of abscission.[6] These
same compounds stimulated the evolution of ethylene only slightly.

The amino acids leucine and valine were reported to be relatively poor
stimulators of abscission.[7] They also had no effect on ethylene produc-
tion during either abscission stage and the abscission response was like-
wise negligible. Methylene endothal and Δ-methylene endothal were not
active on whole bean plants and stimulated explant abscission and ethylene
evolution only slightly.

We conclude, therefore, that endogenously produced ethylene is impli-
cated in the processes of bean leaf abscission. Caution, however, must be
exercised in interpreting the data. The method is limited to measuring
only the observable separation of the tissues, thus making it impossible
to determine if a substance affects the very onset of abscission or only
the final dissolution of the cell wall materials. The possibility also
remains that other substances, both dissolved and volatile, may participate
in initiating leaf abscission.

SUMMARY

Various auxins and nonauxinic compounds were observed for their effects on abscission and ethylene production of bean petiole explants. In general, all substances that accelerate abscission also promote evolution of ethylene. Ineffective abscission stimulators have little effect on ethylene production. It is concluded that endogenously produced ethylene participates in the abscission processes.

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6. Chatterjee, S.; Leopold, A.C. 1963. Auxin structure and abscission activity. Plant Physiol. 38:268-273.

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II. CELL-FREE ETHYLENE EVOLUTION FROM ETIOLATED PEA SEEDLINGS

F.B. Abeles* and Bernard Rubinstein**

Early reports of ethylene evolution from cell-free preparations have been criticized by Burg and Burg.[1]*** Since that time Spencer et al.[3-5] and Gibson[6] have reported on cell-free ethylene evolution. Some of this work has been criticized by Meigh.[7] The unusual property of these preparations to evolve ethylene after exposure to temperatures greater than 90 C compromises their enzymic significance. We will describe a cell-free preparation from peas that evolved ethylene in both an enzymic and nonenzymic manner.

Etiolated epicotyls of 8-day-old peas (Pisum sativum L. var. Alaska) grown on vermiculite at 23 C were harvested and stored in the freezer until used. All procedures were performed between 0 and 4 C. About 250 grams of epicotyls were chopped into small pieces, added to 125 milliliters (ml) of glass-distilled water, and ground in a Waring Blendor until a smooth paste was formed. The paste was squeezed through cheesecloth and the liquid was centrifuged at 12,000 x g for 30 minutes to remove the larger particles.

The enzyme was prepared by adding 11 grams of ammonium sulfate to 50 ml of crude supernatant, centrifuging at 10,000 x g for 15 minutes, and discarding the pellet. An additional 8 grams of ammonium sulfate was added, the solution was centrifuged at 10,000 x g for 15 minutes, and the resultant pellet was taken up in 10 ml of water to be dialyzed overnight against water. The dialyzed protein was then cleared by centrifugation at 10,000 x g for 15 minutes and found to have a protein concentration of about 7 mg of protein per ml as determined by absorption at 260 and 280 millimicrons (mμ).[8] The enzyme prepared by this method appeared to be soluble, because centrifugation at 144,000 x g for one hour did not result in a significant decrease in activity.

It was also possible to prepare an active protein fraction from the original crude supernatant by adding CM-Sephadex C-50 to remove the substances at lower molecular weight. However, the ammonium sulfate method was used for the experiments described here in order to concentrate the protein. The enzyme was stable at 0 C with a 50% loss in activity after two days.

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Substrate was prepared from the original crude supernatant by adding 1.5 volumes of acetone per volume of supernatant to precipitate protein. The precipitate was removed by centrifugation at 10,000 x g for 15 minutes, and the acetone was evaporated off under vacuum at 30 C. This crude substrate was stable at 0 C and lost a negligible amount of activity in three days. It is also possible to prepare substrate by dialyzing the crude supernatant against water and evaporating the resultant dialyzate down to the original volume of crude supernatant. Although identical results were obtained with both procedures, the acetone precipitation method was used as a matter of convenience.

The reaction was run in a 5-ml syringe (liquid volume 2.5 ml, gas volume 2.5 ml) fitted with a rubber vaccine cap so that a 2-ml gas sample could be withdrawn for analysis. The syringes were shaken 80 times a minute with an amplitude of 2 cm at 29 C. Presence of ethylene was determined by gas chromatography by a method described earlier.[9]

Data in Table 1 show that there was no ethylene evolution by the enzyme alone and a slight evolution of gas by the substrate alone. Stepwise increases in protein resulted in similar increases in ethylene evolution. Increasing the amount of substrate while holding the protein concentration constant resulted in an increasing rate of gas production until the process was substrate-saturated. Fifteen minutes were routinely used to determine the rate of ethylene evolution because gas production was linear within this time.

TABLE 1. ETHYLENE EVOLUTION BY CELL-FREE PEA EXTRACTS[a]

Protein, mg	Substrate, ml	Ethylene Picoliters/ 15 min
0.0	1.4	0.10
0.1	1.4	0.30
0.2	1.4	0.60
0.4	1.4	1.70
0.5	0.0	0.0
0.5	0.5	1.1
0.5	1.0	2.1
0.5	1.5	2.7
0.5	2.0	2.70

a. 125 µmoles of acetate buffer (pH 4.6); 0.125 µmole $Mn(NO_3)_2$; 2.5 ml liquid volume; 29 C.

Heating at 100 C for ten minutes destroyed all enzymic activity and at 60 C lowered the activity to half that of the original. The substrate was also found to be heat-labile. The substrate became inactive for enzymic release of ethylene if the pH was raised to 9 for 10 minutes and then lowered to the original pH of 6. Half of its activity was destroyed at pH 7.5. The substrate was stable between pH 6 and 4, but lower pH partially destroyed activity. For example, pH 3 for 10 minutes caused a 25% decrease in activity.

With 50 millimoles of acetate buffer the pH optimum for the reaction was between 4.5 and 4.7. A similar pH optimum was observed with citrate buffer, although the rate was one-fourth that in acetate.

A series of ions were tested for their effect on the reaction, and only manganese (Mn^{+2}) at 5×10^{-6} M stimulated ethylene liberation. Greater concentrations of Mn^{+2} progressively inhibited the reaction. Other ions tested that had either no effect or inhibited in a concentration range of 10^{-2} to 10^{-4} M were Al^{+3}, Ca^{+2}, Co^{+2}, Cu^{+2}, Fe^{+2}, Fe^{+3}, K^+, Mg^{+2}, Mo^{+6}, Na^+, Ni^{+2}, and Zn^{+2}.

The coenzymes adenosine triphosphate (ATP), coenzyme A (CoASH), thiamine pyrophosphate, nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide reduced (NADH), nicotinamide adenine dinucleotide phosphate (NADP), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), at a range of concentrations, had either no effect or inhibited the ethylene-evolving reaction.

The most effective inhibitor tested was cyanide (CN^-), which inhibited evolution of ethylene by 50% at 10^{-4} M. Azide and sodium fluoride (NaF) produced a 50% inhibition at 10^{-3} M; mercury (Hg^{+2}), dinitrophenol, iodoacetate, and hydroxylamine inhibited 30% or less at 10^{-2} M. Ethylene-diaminetetraacetate (EDTA) at a concentration of 5×10^{-4} M resulted in a 50% decrease in ethylene evolution. Addition of larger amounts of Mn^{+2} had only slightly relieved this inhibition.

Compounds containing SH groups also inhibited the production of ethylene. Thioglycolic acid was most effective (100% at 10^{-3} M); thioglycerol, cysteine, reduced glutathione, and CoASH, respectively, had less effect. Ascorbic acid behaved similarly to the compounds mentioned above. In the presence of these compounds our preparations evolved ethane in quantities equivalent to the amounts of ethylene normally produced. All of the above compounds initiated ethane evolution from the substrate alone, but for cysteine, glutathione, thioglycerol, and ascorbate, the presence of protein enhanced the rate of ethane evolution.

Ethanol, ethionine, methionine, glycine, glycolic, glyoxylic acid, ethane, and acetyl-coenzyme A were added to the enzyme in the presence of a limiting amount of substrate to see if they were possible precursors of ethylene. None had any stimulatory effect.

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Although indoleacetic acid stimulated ethylene production in intact plants,[9] it had no effect on gas production from cell-free preparations.

In addition to the enzymic production of ethylene from the substrate, it was found that iron Fe^{+2} ions and flavin mononucleotide (FMN) ($5x10^{-3}$ M) would cause ethylene evolution from the substrate alone at a rate greatly exceeding (sevenfold for FMN and fourfold for Fe^{+2}) that obtained with a saturating amount of enzyme. In addition, the total amount of ethylene evolved in the nonenzymic reaction was greater than that in the protein-mediated reaction.

Thus it appears that ethylene evolution from cell-free preparations of etiolated peas can be mediated by both enzymic and nonenzymic means; this may explain in part some of the conflicting reports of earlier workers. It should be possible to determine the compound involved in the biosynthesis of ethylene for the system described in this paper.

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III. ENDOGENOUS ABSCISSION INDUCERS: RECENT PROGRESS

F.T. Addicott and O.E. Smith*

The research program of the group at Davis is directed to isolation and determination of chemical structure and physiological properties of the endogenous substances (hormones) that affect abscission of plant organs. The major substances known to have important influences on abscission include abscisins, auxins, gibberellins, kinins, and ethylene. This paper reports some of our recent work with abscisin II and with indole-acetic acid (IAA), the principal endogenous auxin.

A. ABSCISIN II

Substantial progress was made during the past year in the difficult chemical work of synthesizing the molecule postulated for abscisin II. By the close of the year a structure was synthesized that differed from abscisin II only by the lack of two H atoms, having a triple bond where a double bond should be. The synthesized compound showed abscission-accelerating activity equivalent to that of abscisin II (Figure 1A).

The parallel work of isolating a new supply of abscisin II from natural sources required a great deal of time, mainly because of the large volumes of solvents and other reagents required. Twenty-eight 55-gallon drums of acetone alone were used in the initial extraction of the 2,100 pounds of young cotton fruit. In addition, ten 55-gallon drums of ethyl acetate were used during purification by acid-base fractionation. Further purification, using seven different separations by either column or paper chromatography resulted in a yield of 10 milligrams (mg) of crystalline abscisin II. This amount will permit a number of important experiments further characterizing the physiological properties of abscisin II, but will not permit much further exploration of the chemical properties.

Some abscission effects of applications of pure abscisin II to cotton seedling explants (excised abscission zones) are shown in Figure 1. Abscission acceleration from distal applications (to petiole stumps) of 1.0 and 0.1 microgram (μg) is greater than can be obtained from maximum accelerating concentrations of gibberellin A_3 (GA_3) or IAA. Abscisin II is also active when applied proximal (to stem stumps) to the abscission zone, but appears to be somewhat less effective.

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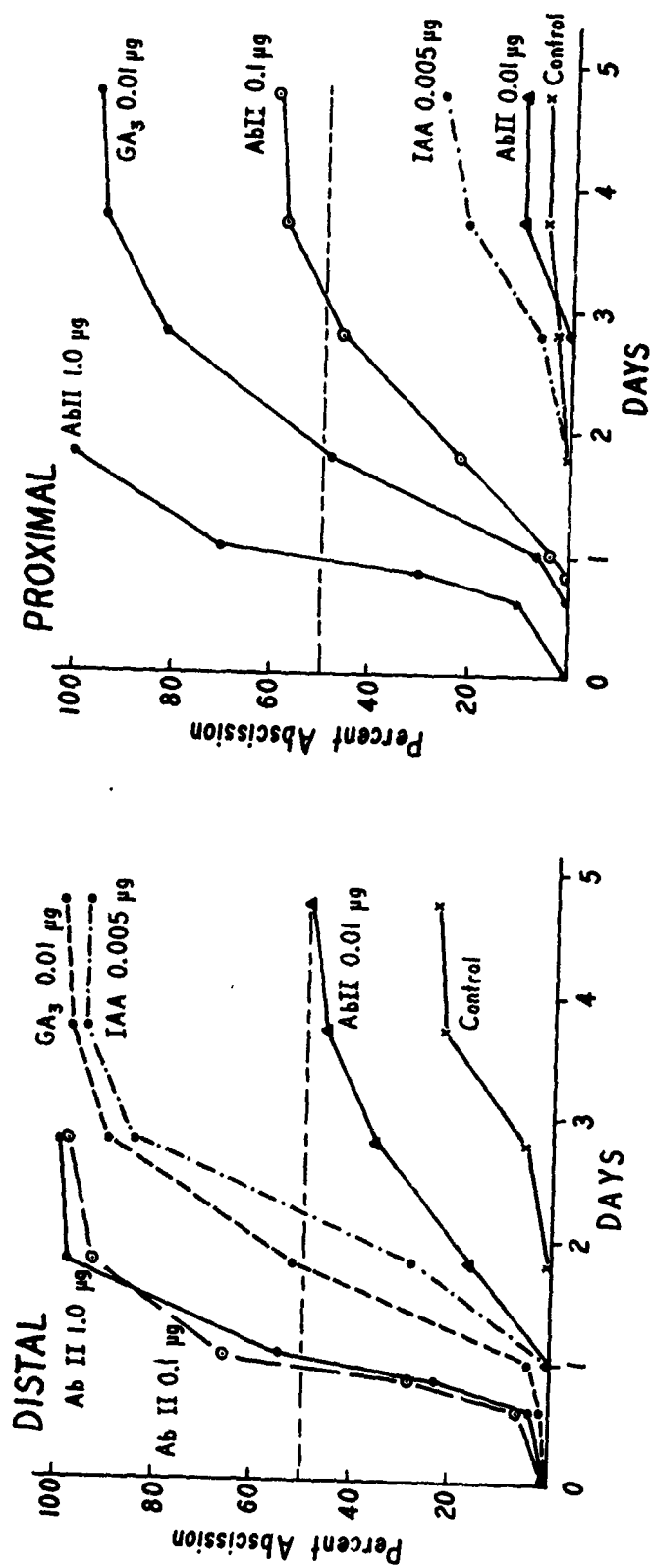
Figure 1. Abscission Acceleration Induced by Pure Abscisin II. Amounts of 1.0, 0.1, and 0.01 microgram were applied to petiole stumps (distal application) or to the stem stump (proximal application) of explants (excised cotyledonary nodes) of cotton seedlings. Each treatment included 30 explants (60 abscission zones). The curves show the time-course of abscission. For comparison, a distal treatment with an optimum amount of GA_3 is included.

A. Early onset and rapid rate of abscission induced by abscisin II.

B. Proximal applications of 0.1 and 0.01 microgram were considerably less effective than the corresponding distal applications.

Abscisin II is a strong inhibitor of growth in the classical _Avena_ coleoptile test (straight growth), as illustrated in Figure 2B. The data of Figure 2A, obtained with an impure preparation, show a very different concentration response, indicating the necessity of isolating endogenous substances in pure form before their properties can be accurately determined.

B.   AUXIN

The literature shows that auxin can either accelerate or retard abscission, depending on a number of factors including (i) growth status of tissue, (ii) amount of tissue used, (iii) site of application, (iv) amount applied, and (v) time of application in relation to excission. Most research publications in the field have reported experiments with only one or two of these factors. Clearly, to understand the function of auxin in the control of abscission, it will be necessary to analyze the interaction of all such factors. As one step in this direction, a range of amounts of IAA was applied to explants of cotton seedlings, either distal or proximal to the abscission zone or in various combinations.

The results of distal applications alone and of proximal applications alone are shown in Figure 3. The distal applications retarded and inhibited abscission; the proximal applications accelerated it. Other experiments showed that when the amounts of proximal IAA are increased, the rate eventually falls and ultimately complete inhibition is obtained. The curves in Figure 3 illustrate the basic pattern of response of the cotton explant material to applied IAA. However, as the experimental factors are varied the curves shift. One such shift is shown in Figure 4, where the control curve is that for proximal applications alone and the experimental curve resulted from the application of a range of amounts of IAA proximally with 100 milligrams per liter applied distally in all cases. As the amount of proximal IAA increased, the response changed from strong retardation to a significant acceleration. This is a striking example of the effectiveness of the gradient of applied auxin (i.e., the site of application) in the control of abscission. Similar response curves were obtained with distal applications of 25 and 50 mg per liter.

Examination of the data of these experiments disclosed no consistent relationship between the total amount of auxin applied and the rate of abscission. On the other hand, strong accelerations and retardations were correlated with the relative amounts of auxin applied proximally and distally. If the amount applied proximally was greater than that applied distally, abscission was accelerated; if the amount distally was greater than that applied proximally, abscission was retarded. Selected data that illustrate these correlations are shown in Figure 5. This graph may be described as multiphasic response curve of abscission to increasing amounts of auxin.
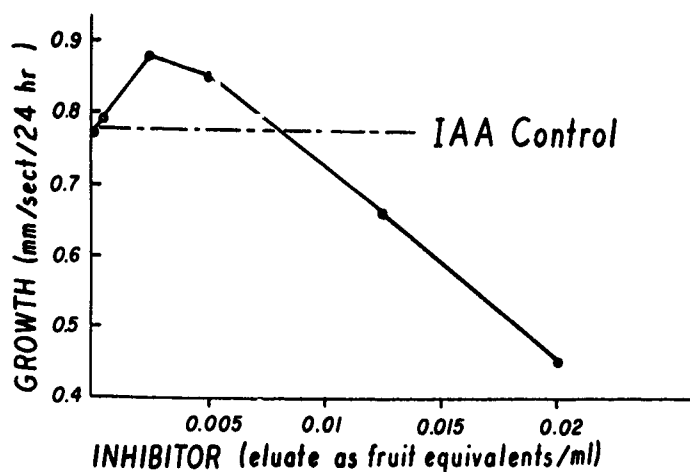
A
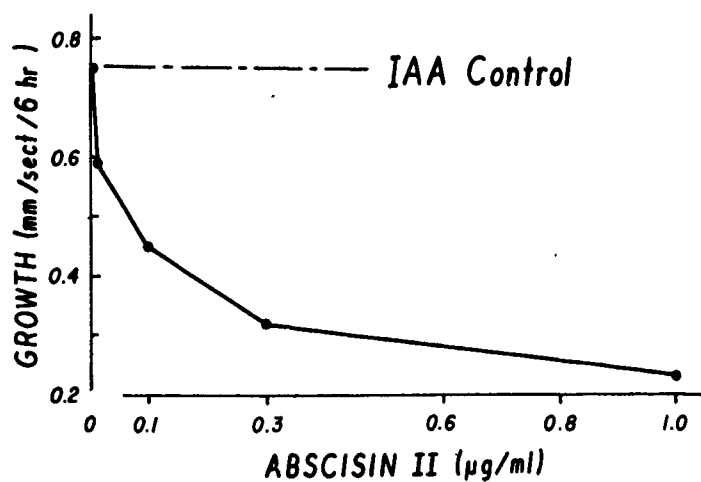
B

Figure 2. Effect of Inhibitor Eluate and Abscisin II on Growth
of <u>Avena</u> Coleoptile Sections.
A. Inhibitor eluate. Points show average growth of
60 sections, initial length 3.2 millimeters. Petri
dish contained ten sections in 20 ml of basal medium
with 0.1 μg/ml IAA, pH adjusted to 6.5.
B. Abscisin II. Points show average growth of 20
sections, initial length 5.06 millimeters. Each
tube contained ten sections in one ml of 2% sucrose,
0.1 μg/ml IAA, buffered at pH 4.8.
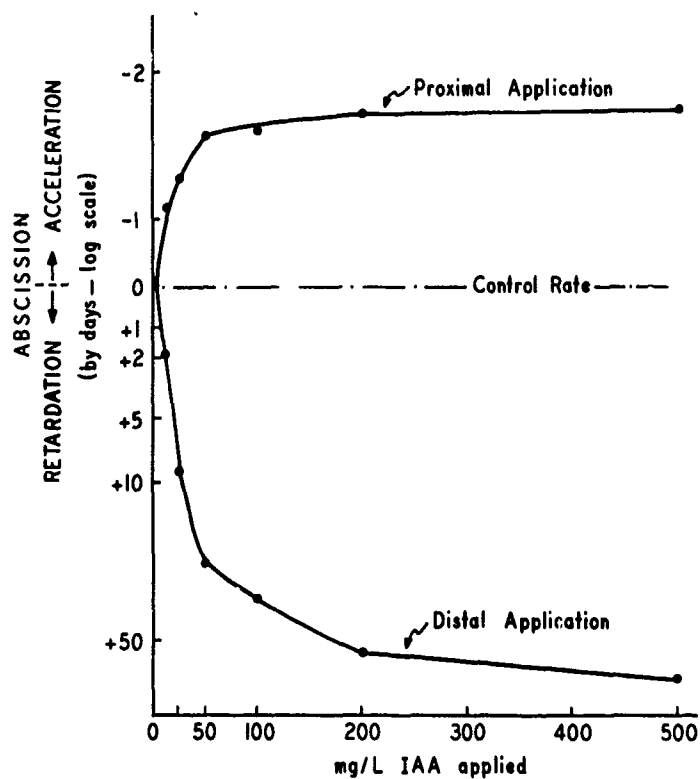
Figure 3. Rate of Abscission as Affected by Application of Auxin (IAA) to Either Petiole Stumps (Distal) or to Stem Stumps (Proximal) of Cotton Explants.

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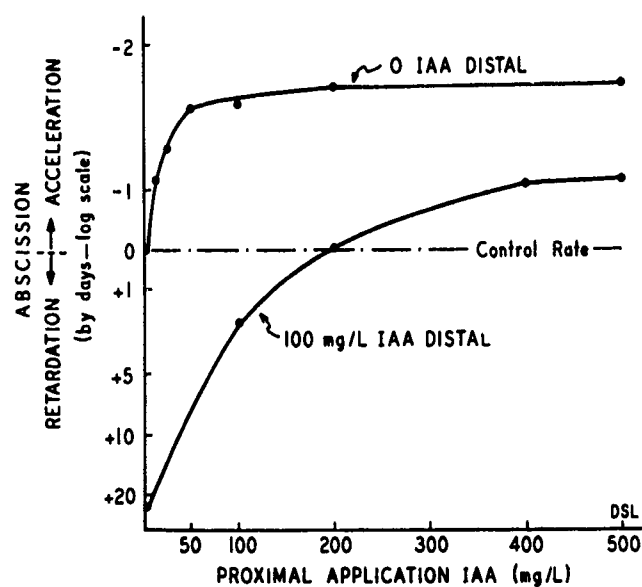
Figure 4. Effect on Abscission of Applying
Various Amounts of IAA Proximally
in Combination with a Constant
Amount of IAA Applied Distally.
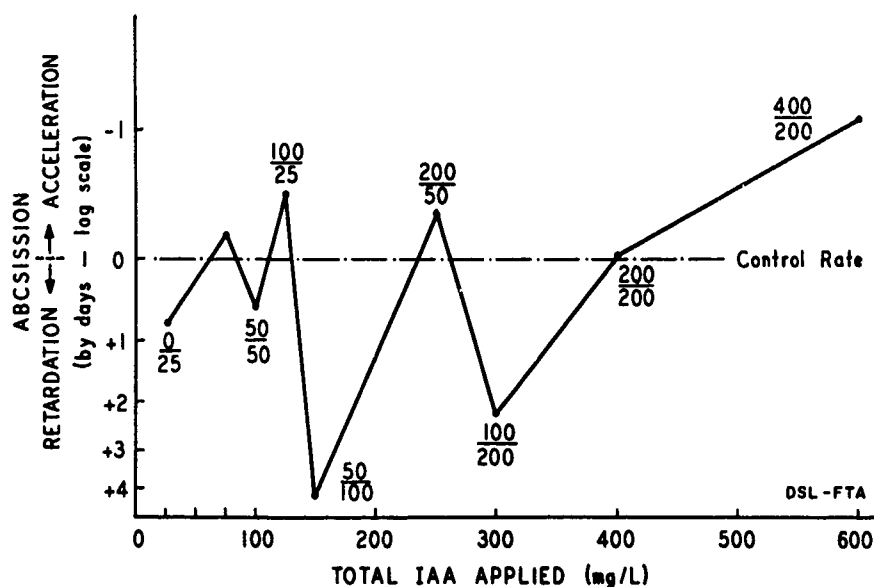
Figure 5. The Multiphasic Response of Abscission to Increasing Amounts
of IAA. Acceleration and retardation are correlated with the
relative amounts of IAA applied distally (the bottom number
of the fraction) and proximally (the top number of the fraction).

IV. MORPHOLOGICAL STUDIES OF BEAN EXPLANT ABSCISSION

Peter C. Scott, Barbara D. Webster and A.C. Leopold*

A. INTRODUCTION

This morning I would like to discuss some of the current research on bean abscission at Purdue University. Since our knowledge of hormonal regulation of abscission has recently advanced quite markedly, we are attempting to correlate the hormonal changes with morphological, metabolic, and biochemical changes in the abscission zone.

Abscission in general has been shown in numerous instances to be influenced by auxin. Two theories have been proposed to explain the auxin effects. The auxin gradient theory states that as long as there is a greater amount of auxin distal to the zone, abscission does not occur, but as the leaf begins to senesce and the auxin concentration on the distal side decreases relative to the proximal side, abscission of the leaf blade occurs.

An alternative theory holds that auxin inhibits abscission if the quantity is high enough, whereas low concentrations promote abscission. Thus, Gaur and Leopold[**] and Biggs and Leopold[2] showed that low concentrations of napthalene-acetic acid (NAA) promoted abscission whether applied distally or proximally, but high concentrations inhibited abscission.

Later work showed in addition that bean explant abscission comprises two distinct stages with reference to time. A high concentration of NAA inhibited abscission if applied immediately or up to 12 to 24 hours after severing the leaf blade from the petiole. If the explants were placed on plain agar for the 12- to 24-hour period and then transferred to a high concentration of NAA, abscission was promoted. Therefore, auxin can regulate abscission not only by the amount at the abscission zone, but by the differential effects it has on two consecutive stages in abscission development.

The first stage only is inhibited by auxin, and the promotions by auxin seem to affect only the second stage. When a bean leaf blade is cut off, its petiole will pass from the inhibited first stage to the second or promotive stage in about 12 to 24 hours. Rubinstein and Leopold[3] suggested, therefore, that the promotions and inhibitions were quite different actions by auxin, and the changeover occurs between 12 and 24 hours after deblading. Our research is oriented to ask what changes in the morphology or biochemistry of the petiole may represent such a change in hormonal regulation.

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** See Literature Cited, page 43.

As experimental variables, we are using promotive and inhibitory treat-
ments with auxin, and also promotions by ethylene gas and inhibitions by
the respiratory inhibitor dinitrophenol (DNP).

B. METHODS

Seeds of Phaseolus vulgaris were sown in vermiculite and germinated
under constant environmental conditions. After 15 days, explants one
centimeter long were cut to include the laminar abscission zone of the
fully expanded primary leaves. These explants were placed upright in
petri dishes containing 1% agar and put in the growth chamber for the
various lengths of induction time.

Explants were fixed for subsequent anatomical study in formalacetic
acid-alcohol and aspirated for approximately three hours. The tissue was
then carried through the tertiary butyl-ethyl alcohol series to 100%
tertiary butyl alcohol. This procedure was followed by gradual infiltra-
tion with Paraplast, in which the tissues were finally embedded.

Ten-micron longitudinal and transverse sections of the material were
cut and after staining in safranin and fast green, slides were mounted
in Permount and dried for several hours on a warming table.

C. RESULTS

From the first examination of the sections, one outstanding morphologi-
cal change appears to be correlated with natural abscission and with treat-
ments that promote abscission. In all the sections where abscission took
place, we found tyloses developing in the xylem elements at the abscission
zone. By tyloses we mean the active invagination of a living protoplast
into a nonliving xylem element plugging it against the flow of sap. These
outgrowths, or tyloses, are from living parenchyma cells that surround the
xylem elements in the vascular bundles. This active process would seem
to be an instance of localized growth of the cell wall surface and involve,
in addition, a radical change in the plasma membrane and cytoplasm of the
parenchyma cell. The regulation of tylosis formation may be a possible
mechanism for the regulation of abscission, whether by hormones, ethylene,
or other abscission-regulating chemicals.

Let us first consider the anatomy of the primary bean leaf petiole as
it is cut from a 15-day-old plant. The pith consists of large compactly
arranged parenchymatous cells that are continuous with those of the cortex
through the medullary rays. Seven to ten vascular bundles arranged in a
circle traverse the length of the petiole and coalesce in the laminar
pulvinar region. The most conspicuous elements of the vascular bundle
are the large round metaxylem elements. Xylem parenchyma cells are inter-
spersed among the metaxylem (Figure 1A).

The sieve tubes in the phloem are long and narrow with simple sieve plates in the end walls. The cortical cells are parenchymatous, often containing starch grains and crystals of calcium oxalate. The cortex is limited peripherally by a single layer of epidermal cells that have thickened outer and radial walls (Figure 1B).

If the leaf blade is removed and one-centimeter explants are placed on plain agar for 36 hours, we can see the following anatomical changes occurring. Vessels in the abscission zone are being filled with tyloses and the cortex has conspicuous newly divided cells (Figure 2). An area of cell division activity is present in many instances of abscission but not in all cases. This layer of new cells forms a protective surface after abscission has taken place.

After 72 hours from leaf blade removal, there is extensive plugging of the metaxylem with tyloses (Figures 3A and 4A). Tiers of newly divided cells are evident all across the cortical parenchyma (Figure 4B).

Normal abscission in the explant occurs at about 100 hours and if we compare the morphology of the abscission zone of the old bean (30-day old plants), the similarity is striking. The anatomical changes are the same in the aging intact leaf, although they are progressing more slowly than in the debladed petioles. Here again tyloses block the vessels and some cell division takes place. The actual abscission break is beginning in the old bean leaf (Figure 5).

A striking effect is obtained when abscission is promoted by ethylene or low concentrations of auxin (Figure 6). In each case as abscission is promoted so also is the development of tyloses in the vessels. Following chemical treatments that inhibit abscission, there is no evidence of tylosis formation. This is true in petiole explants inhibited by high auxin concentrations or by DNP (Figure 7).

These results would seem to suggest a definite involvement of tylosis formation in the hormonal regulation of bean leaf abscission.

D. SUMMARY

Tylosis formation has been found to be positively correlated with normal and accelerated bean explant abscission. Treatments that promote abscission retard tylosis development. It is suggested that plugging the conductive cells in the region of the abscission zone may be specifically related to the separation processes.
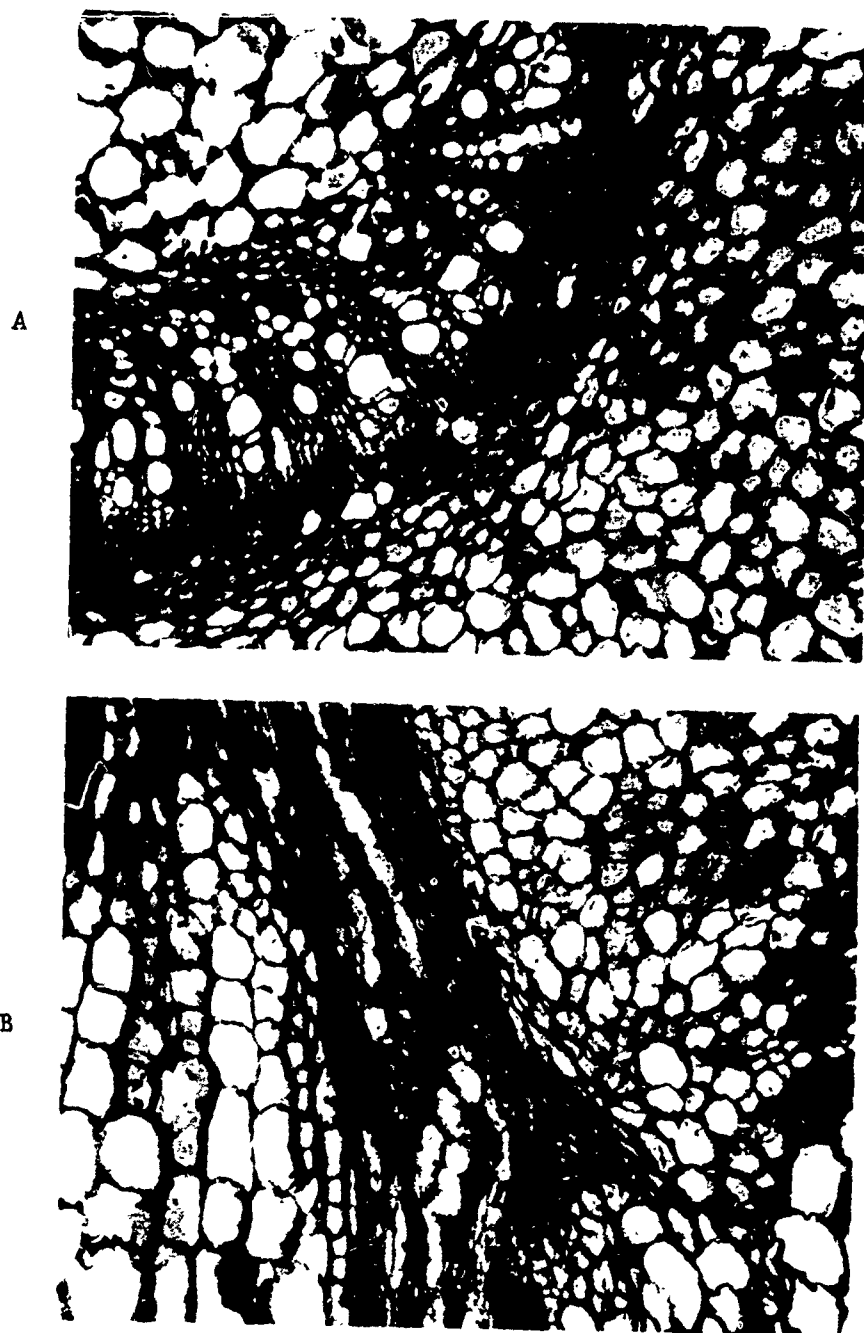
Figure 1. Sections of Primary Leaf Petiole of __Phaseolus vulgaris__ Showing No Induction.
  A. Cross section. Completed arc of vascular tissue above abscission region. Thickened walls of cells at edge of bundle may be pericycle.[4] All vessels clear.
  B. Sagittal section. Epidermal invagination at extreme right edge. Metaxylem elements short, chunky, and clear. No evidence of actual cell divisions in cortical parenchyma, nor any tyloses in vessels.
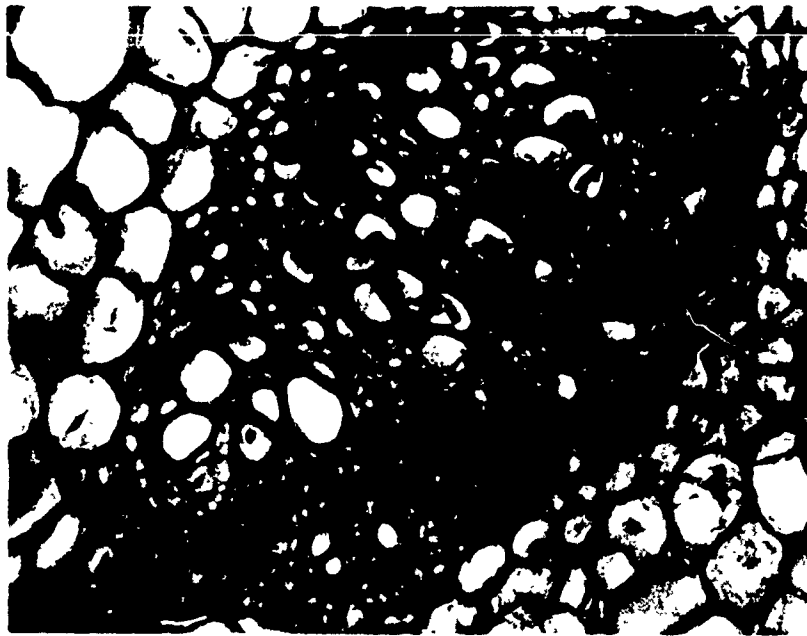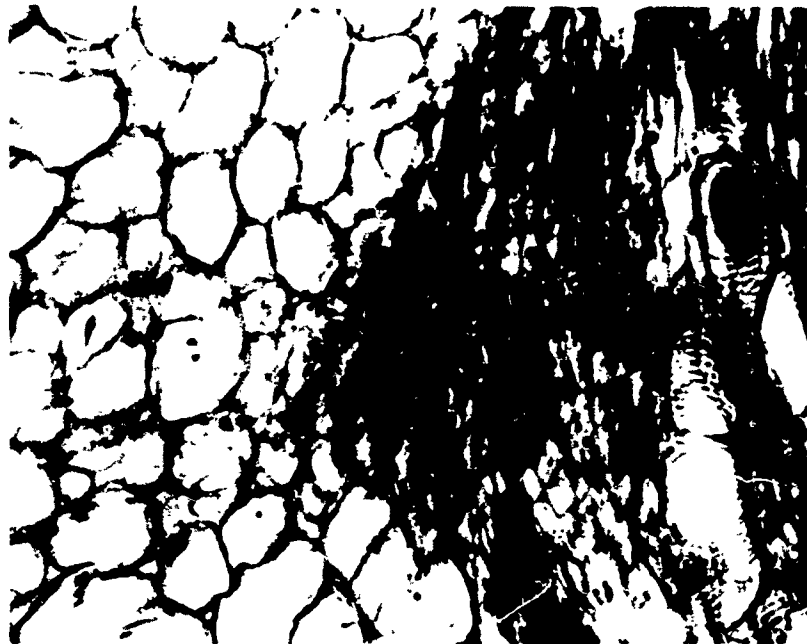
Figure 2. Sections Showing Anatomical Changes after
Leaf Blade was Removed and One-Centimeter
Explants Placed on Plain Agar for 36 Hours.
A. Cross section. Cortical cells dividing,
vessels filled with tyloses, cortical
cells nucleate.
B. Paradermal section. Anaphase in a cortical
cell, short metaxylem vessel containing a
large tylose.
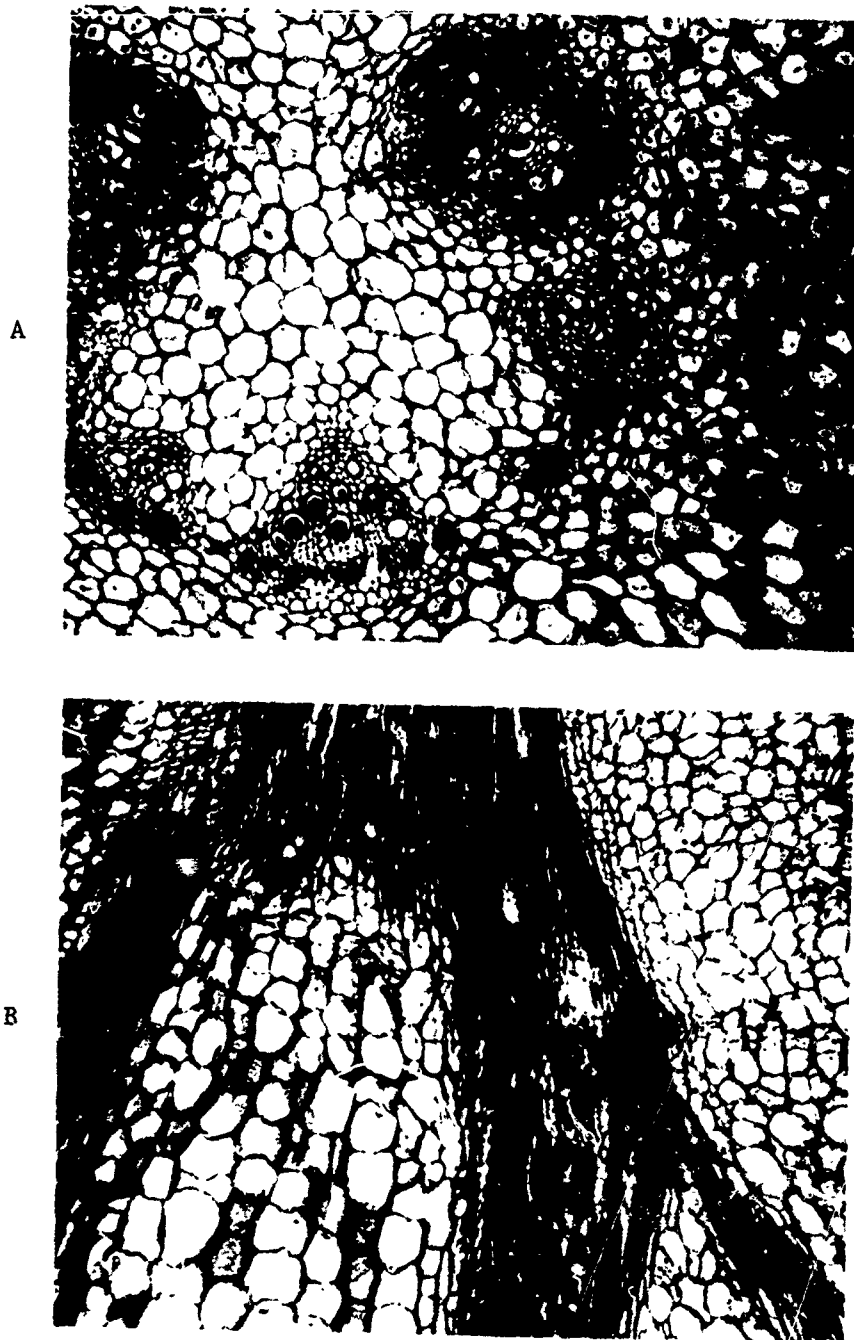
Figure 3. Sections Showing Anatomical Changes after
Leaf Blade was Removed and One-Centimeter
Explants Placed on Plain Agar for 72 Hours.
A. Cross section. At the area of abscission
vascular bundles are coalescing, cortical
cells are dividing, and metaxylem elements
are filled with tyloses.
B. Sagittal section. Tiers of newly divided
cells in the cortical and pith parenchyma.
Evidence of tyloses both above and below
abscission zone. Cell divisions may con-
tinue into vascular parenchyma.
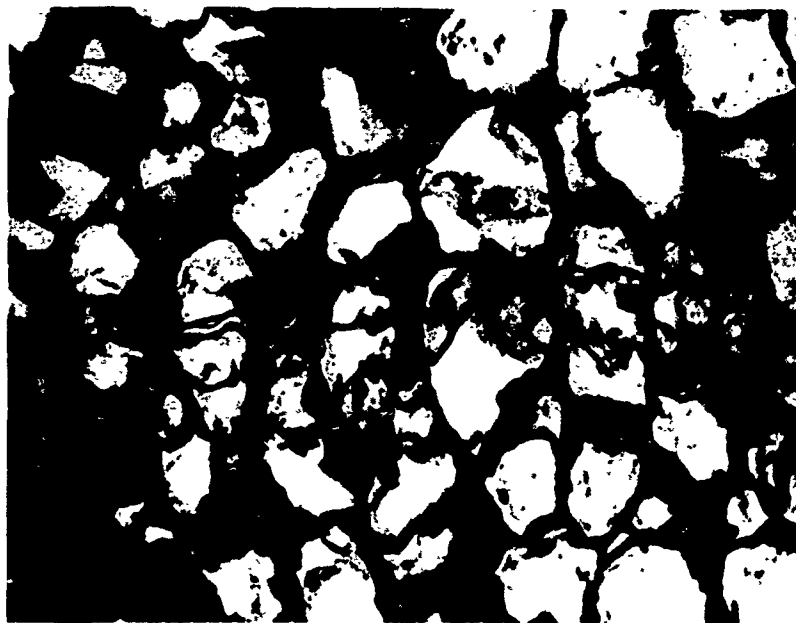
Figure 4.   Sagittal Sections Showing Anatomical Changes after Leaf Blade Removal and
            One-Centimeter Explants Placed on Plain Agar for 72 Hours.
            A.   Tylose material in vessels. Nuclei also appear in vessels as
                 further evidence of tyloses.
            B.   Tiers of newly divided cells across the cortical parenchyma at
                 the abscission zone. Uppermost cell in one tier is at metaphase.
                 Newly divided cells are retained within the mature cell wall.
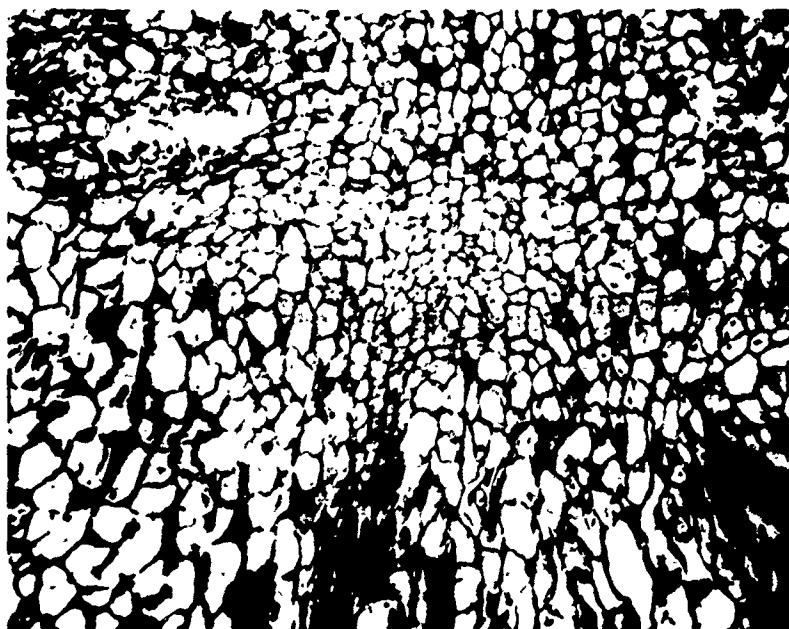
Figure 5. Sagittal Section. Very old, naturally aged.
Extension of newly divided cells across the
width of the petiole and beginnings of the
actual breaking of cells (to the left). Some
vessel blockage in the fragment of bundle of
vascular tissue at lower part of photograph.
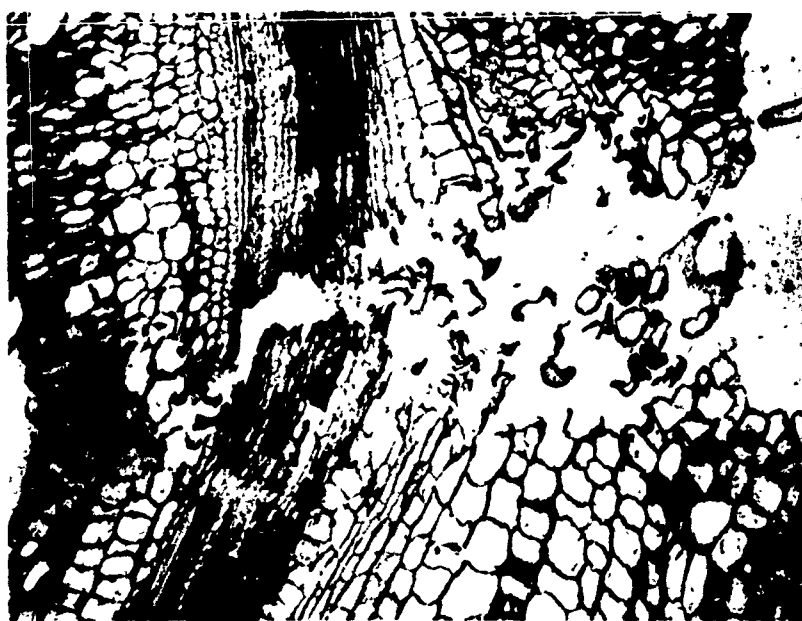
Figure 6. Sagittal Sections. Abscission promoted by ethylene or low concentration
of napthaleneacetic acid (NAA).
  A. Ethylene treatment. Actual break from the adaxial to the abaxial
     side of the petiole. Some cells are completely broken, and some
     intact cells are broken away. Vessels are partially blocked with
     tyloses.
  B. NAA treatment. Vessels filled with tyloses. Limited cell division.
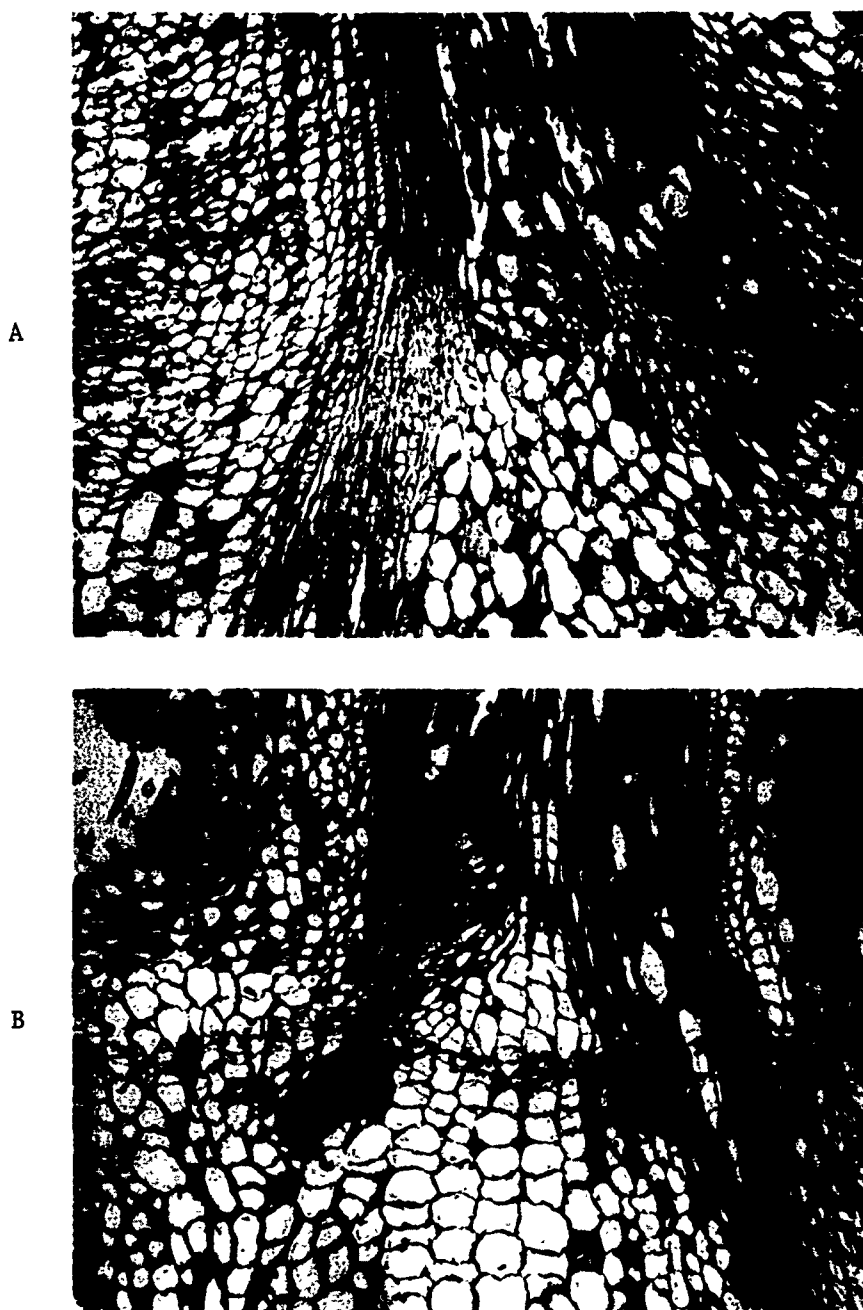
Figure 7. Sagittal Sections. Abscission inhibited by high concentrations of auxin
or by dinitrophenol (DNP). Xylem vessels clear, no evidence of tyloses.
A. Napthaleneacetic acid (NAA) treatment. No evidence of cell division.
B. DNP treatment. Tiers of dividing cells extending across the width
of the abscission zone.

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V. CELL WALL-CYTOPLASM INTERACTIONS ASSOCIATED WITH LEAF ABSCISSION

D. James Morré and A.C. Leopold*

A. INTRODUCTION

Electron microscopy reveals a marked alteration in appearance of membrane structures in cells of the abscission zone.** As abscission proceeds the cell membrane, chloroplasts, mitochondria, endoplasmic reticulum, and other membrane structures can be observed in increasingly severe stages of degeneration. At the time of cell separation, the plasma membrane appears ruptured and is often reduced to a series of wall-associated remnants. Cell wall breakdown also becomes evident, including an apparent dissolution of intercellular cementing substances.

A clue to the nature of the cell wall changes has come from measurement of the relative extractability of cell wall fractions as abscission proceeds (Fig. 1). With increasing time after deblading, both cold and hot water-soluble carbohydrate fractions increase, whereas fractions available to extraction with dilute acid decrease. These two extraction media would be expected to extract carbohydrate differentially on the basis of degree . of polymerization and cross linking within the cell wall. That acid-extractable carbohydrates decline is evidence that the abscission process may be, in fact, associated with cell wall depolymerization and dissolution.

The auxins, 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-acetic acid (IAA) are known to influence the properties of plant cell walls and to alter the course of abscission as well. The immediate cell wall changes are expressed in the form of an auxin-increased deformability component under externally imposed load. Such differences can be measured using either standard fiber testing techniques*** or tissue bending methods.**** The chemical nature of the auxin-induced wall alteration is not known with certainty but may also involve degradative cell wall changes.[3] That 2,4-D does affect deformability of bean petiole tissue in the vicinity of the abscission zone is shown in Figure 2.

** Christie, A.; Leopold, A.C.; Chambers, J.E.; and Morré, D.J.
   Unpublished work.

*** Olson, A.C.; Bonner, J.; and Morré, D.J. Mechanical properties of
    Avena coleoptiles: I. Changes induced by indole-3-acetic acid.
    Planta (in press).

**** See Literature Cited, page 54.

ABSCISSION ZONE OF
PHASEOLUS VULGARIS

MG CARBAZOLE CARBOHYDRATE, AS ANHYDROGALACTURONIC ACID,
PER GRAM DRY WEIGHT

COLD BUFFER EXTRACTABLE

HOT WATER EXTRACTABLE

ACID EXTRACTABLE

HOURS AFTER DEBLADING
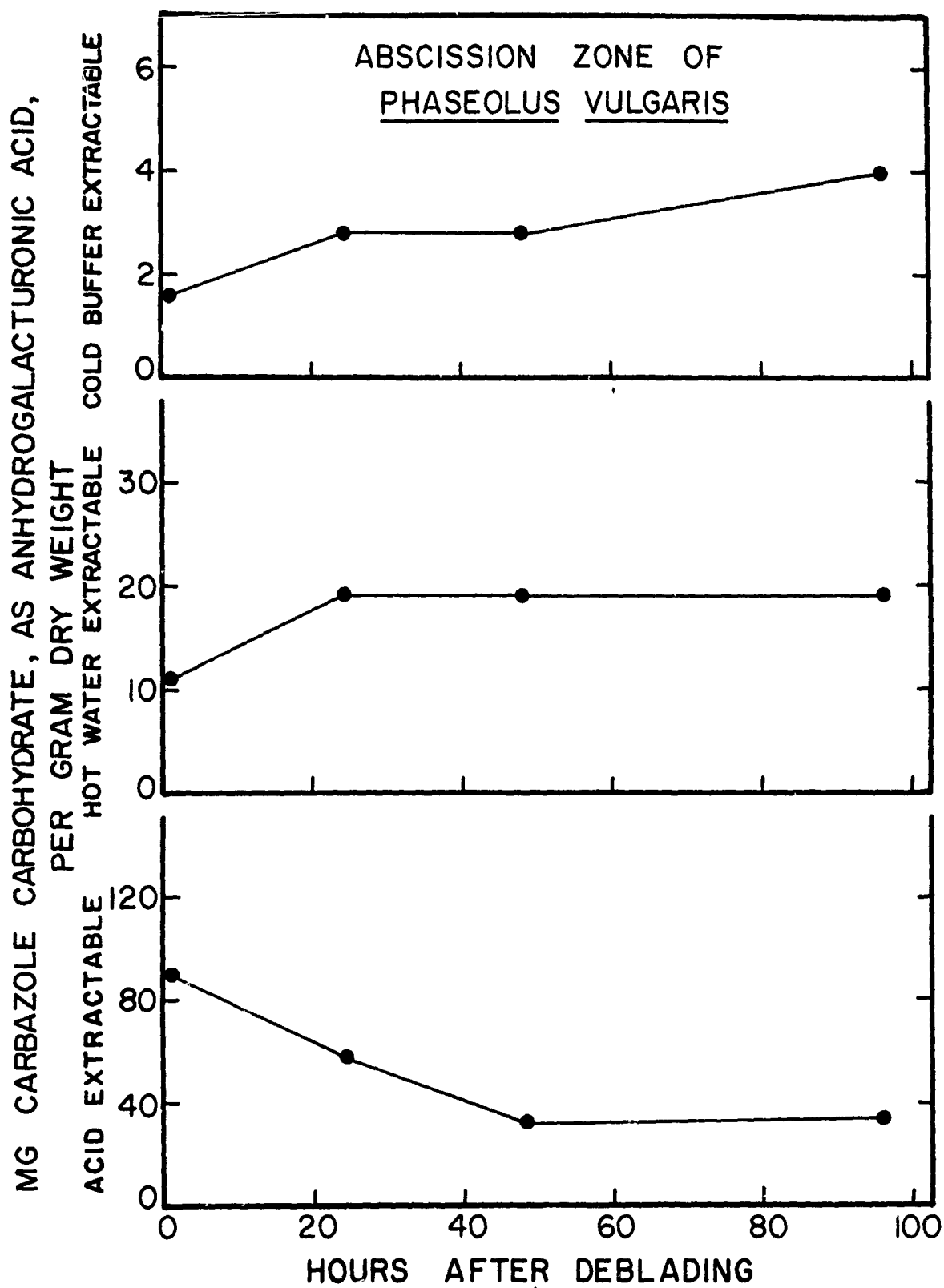
Figure 1. Changes in Bean Explant Abscission Zone Cell Wall
Fractions During Abscission.
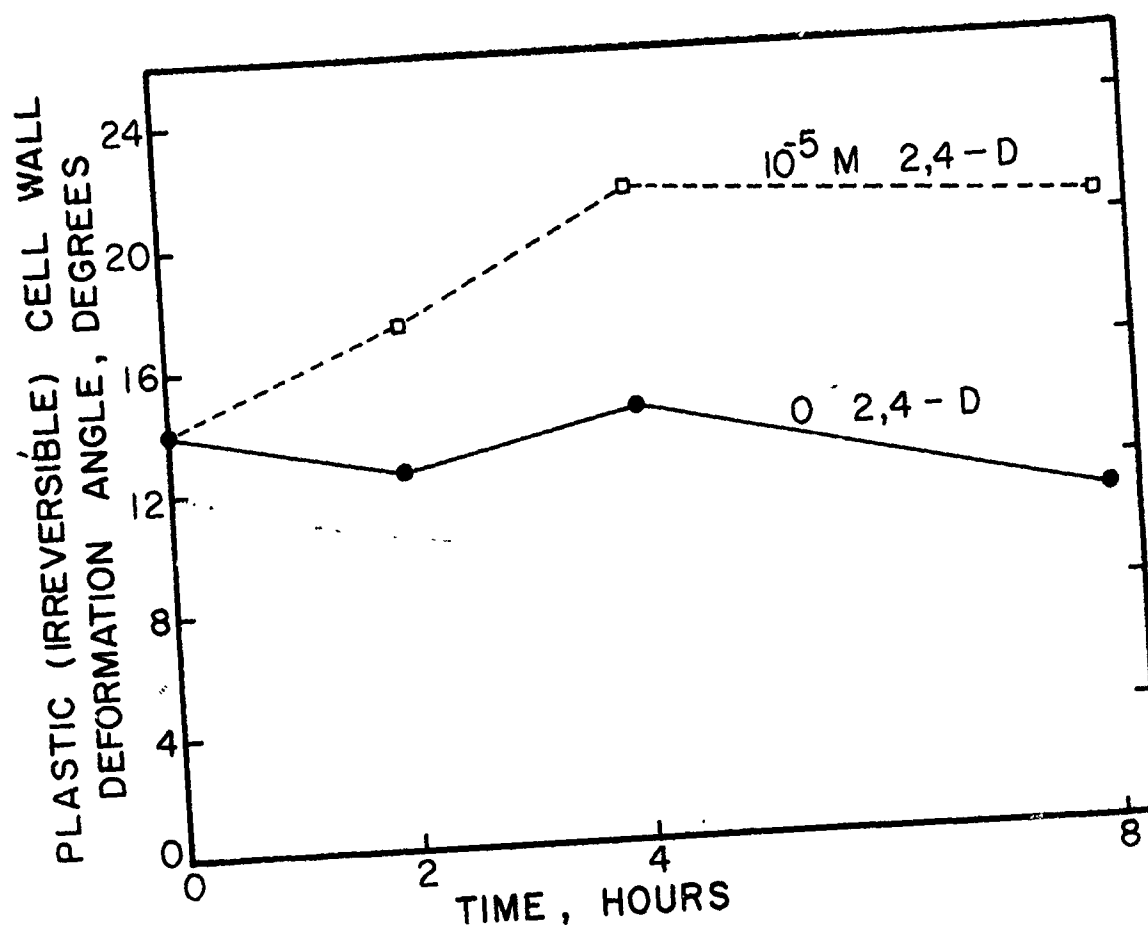
Figure 2. Cell Wall Deformability of 2-cm Bean Petiole Sections Treated with and without 2,4-D.

Of immediate concern, however, is the problem of relating the above cell wall changes to an activity of the cytoplasm. Specifically, we wish to consider the possible involvement of lipoprotein structures in the region of the cell wall - cytoplasm interface (cell membrane) and to relate this information to the functioning of enzymes formed in the cytoplasm.

B. EFFECT OF CHLOROFORM, A LIPIDE SOLVENT, ON PLANT GROWTH AND ABSCISSION

Since membranes are composed chiefly of lipides and proteins, the effect of a known lipide solvent on the abscission process was tested as a first step in establishing a relationship between membrane alterations and leaf abscission. A dilution of approximately one part chloroform in 400,000 parts of air markedly increased leaf abscission as measured by the bean explant test of Rubinstein and Leopold.[3] In the experiments summarized in Figure 3, this represents a shortening of the abscission process by 24 to 48 hours. Control plants required about 96 hours to achieve 50% abscission.

Lower concentrations of chloroform were less effective. A concentration tenfold higher than optimal retards abscission but without total loss of membrane integrity. Explants treated with concentrations of chloroform that were toxic and resulted in bleaching of chlorophyll and/or total loss of turgor failed to abscise. This bimodal effect of chloroform is also evidenced in the ability of chloroform either to promote or inhibit plant growth as a function of concentration (Fig. 4) in much the same manner as auxins.

C. RNA SYNTHESIS, AN ESSENTIAL COMPONENT OF THE ABSCISSION PROCESS

If abscission were simply a matter of making membranes leaky so that cell wall destroying enzymes could escape in the manner of lysosome action, one might have expected that tissue treated with a lipide solvent would begin to abscise as soon as membrane integrity was destroyed. Since solvent treatment resulting in rapid bleaching and loss of turgor actually prevented abscission, other cell processes, such as the synthesis of enzymes, must also constitute a necessary part of the over-all abscission process. Thus, treatment of bean explants with the inhibitor actinomycin D, even for a very brief period, greatly retarded abscission (Table 1).
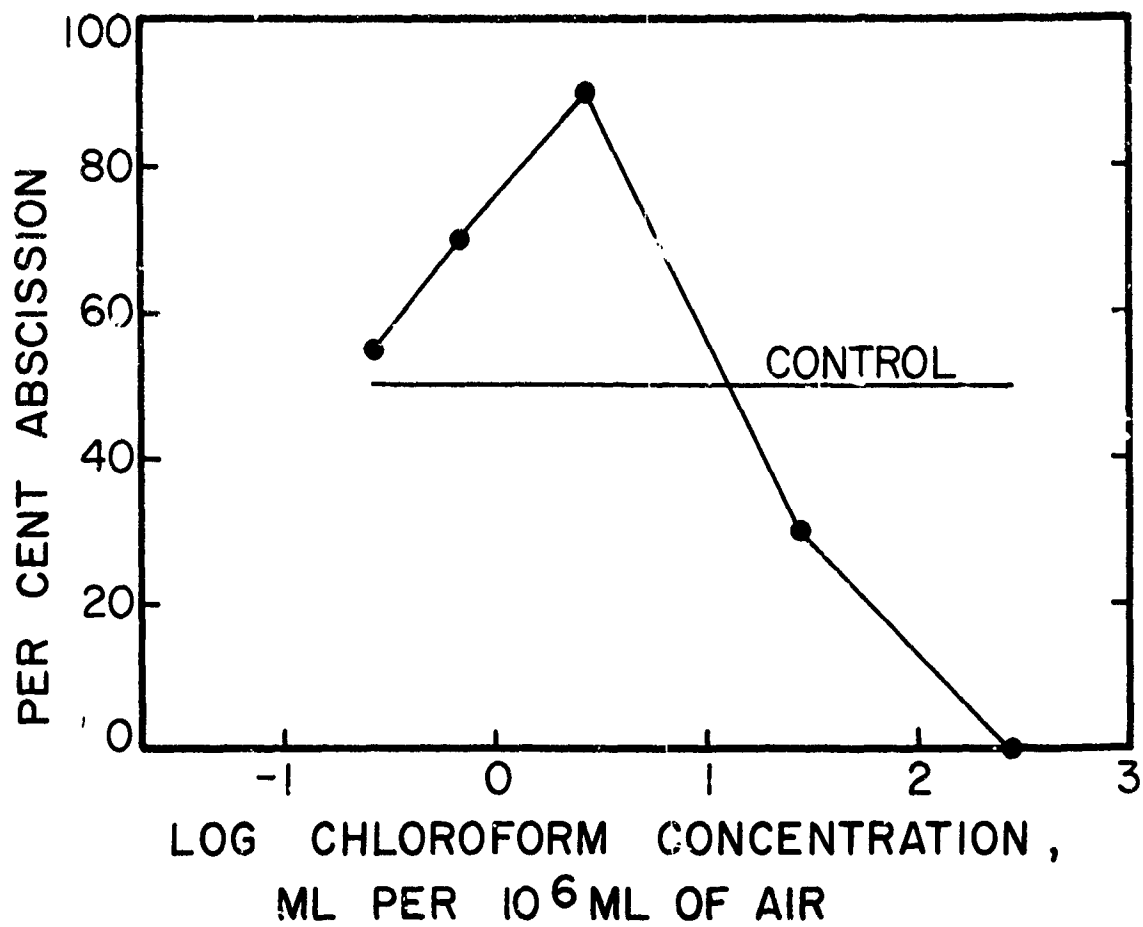
Figure 3. Bean Explant Bioassay Showing Effect of Chloroform Vapor on Abscission.
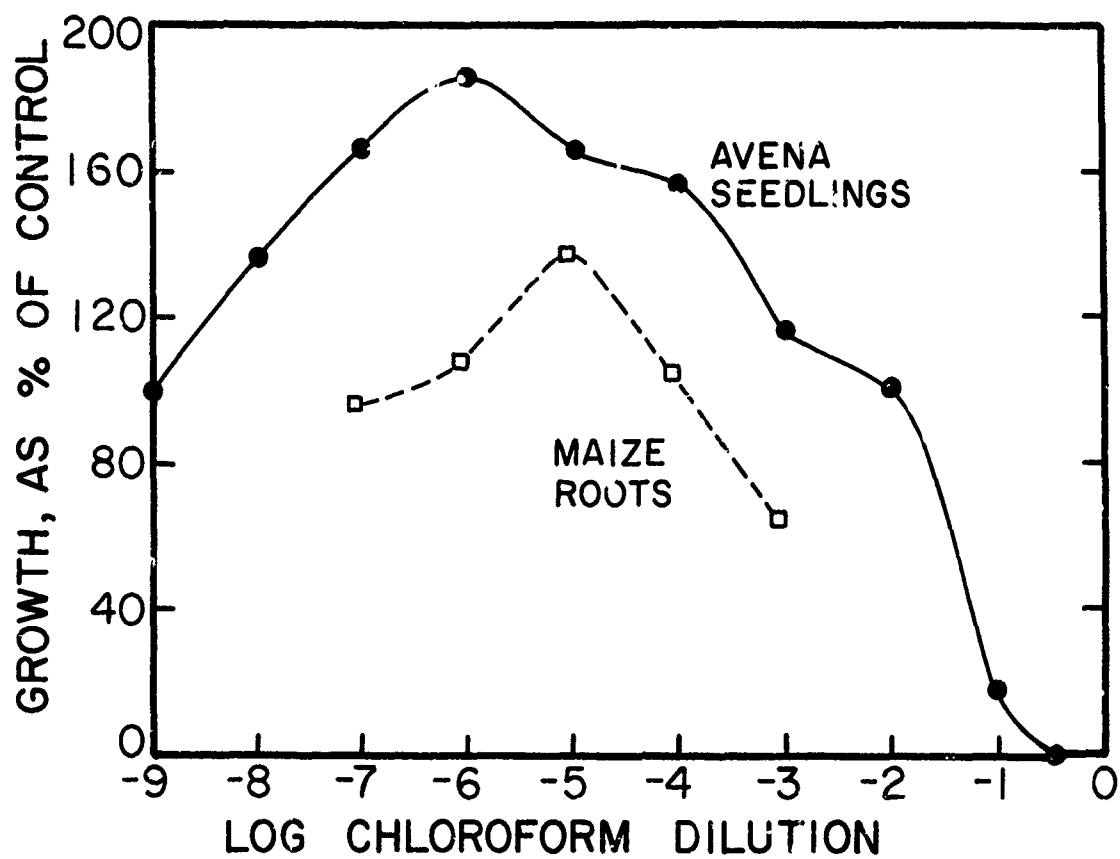
Figure 4. Effect of Chloroform Vapor on Plant Growth. Data
of Morre' and Rogers (in preparation), and of Ber
and Moskwa.[4]

TABLE 1. EFFECT OF ACTINOMYCIN D (SEVEN MICROGRAMS
PER MILLILITER) PRETREATMENT ON ABSCISSION
AS MEASURED BY THE BEAN EXPLANT BIOASSAY

Actinomycin D, pretreatment time	Abscission after 51 Hours, %
0	50
5 minutes	10
1 hour	5
2 hours	0
4 hours	0

Actinomycin D specifically blocks DNA dependent RNA synthesis[5] and,
in so doing, prevents new coding for synthesis of enzymes. Many actino-
mycin D treated explants failed to abscise during the five-day observa-
tion period, whereas, control explants attained 100% abscission in less
than 84 hours.

D. EXPERIMENTS RELATING TO THE ROLE OF AUXIN IN THE ABSCISSION PROCESS

The effect of auxin on abscission is also two-phase with high concen-
trations of auxin preventing abscission and low concentrations of auxins
enhancing abscission when applied to the proximal side of the abscission
zone.[8,8] Electron microscopy has revealed no major structural changes in
the cytoplasm as a result of auxin treatment.* However, auxins may exert
effects on solute uptake including phosphate ion (Fig. 5). Such a process
would presumably involve at least the plasma membrane. The unusual con-
centration dependency of the phosphate uptake response to 2,4-D does not
appear to be the result of simple anion competition. Cell size is also
not a major factor since the basal soybean hypocotyl tissue utilized
increases in fresh weight by less than 2% as a result of 2,4-D treatment.
Under these same conditions, efflux of P^{32} from prelabeled tissue was
increased by about 20% during the same time interval at either 10^{-7} M
or 10^{-4} M 2,4-D.

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* Morré, D.J., and Mollenhauer, H.H. Unpublished data.
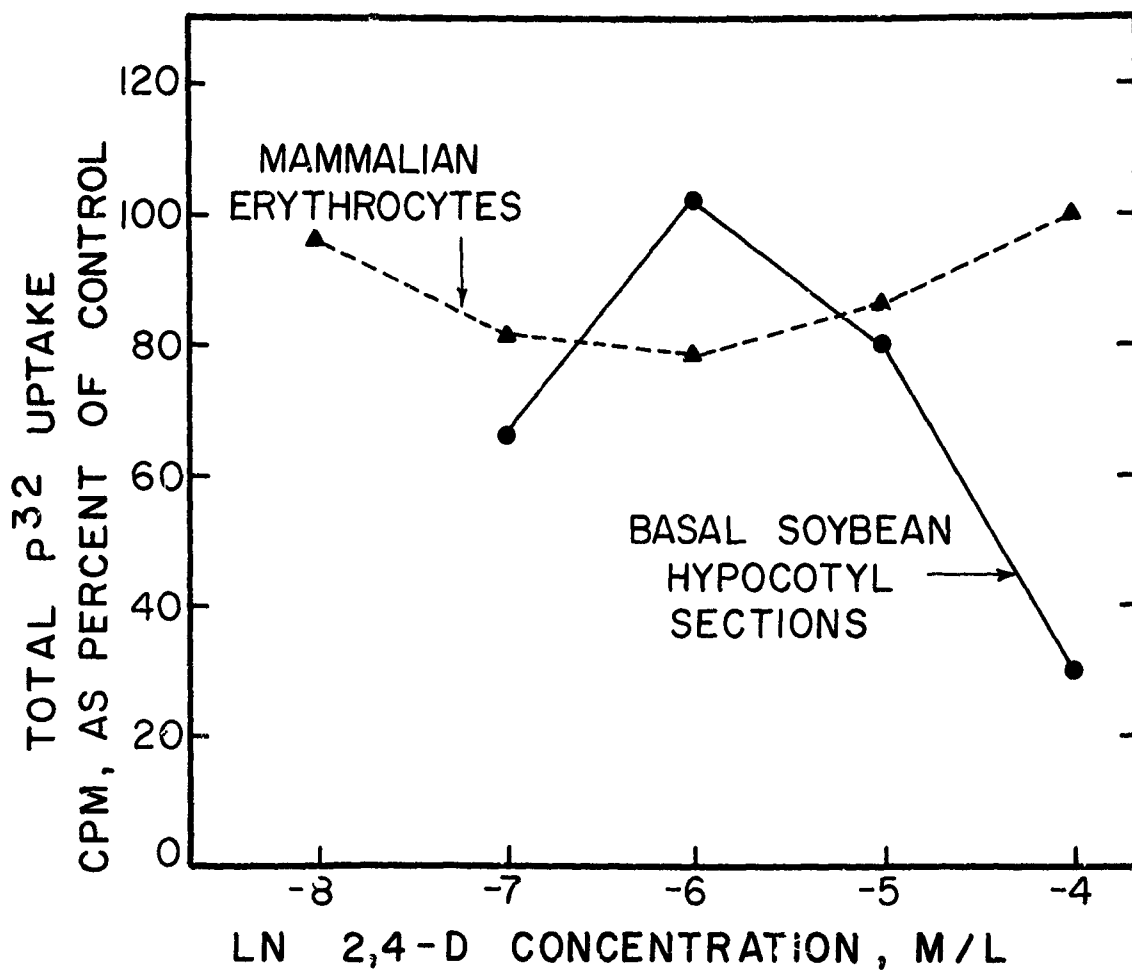
Figure 5. Influence of 2,4-D on Uptake of P^{32} Orthophosphate.
Incubation time: soybean tissue, 4 hrs;
erythrocytes, 45 min.

Mammalian erythrocytes were chosen as a possible model system to permit further study of 2,4-D-membrane interactions. With erythrocytes, the cell contents, chiefly hemoglobin, are surrounded by a cell membrane without the additional complication of an organized cell wall and permanent cell contacts. Also, erythrocytes are without nuclei and do not retain the capacity for active protein or RNA synthesis. In the experiments thus far completed, erythrocytes respond to added 2,4-D by an altered rate of phosphate uptake with a maximum effect of about 20% inhibition. It is not known whether this represents a decreased rate of uptake or an increased rate of efflux.

That auxins may influence cell wall deformability in a manner independent of growth and total RNA synthesis has also been shown.* For this purpose, pea epicotyl sections were treated 4 hours in the presence and absence of 10 to 12 μg/ml of actinomycin D. The sections were then incubated an additional period of 1 to 4 hours with and without the addition of 10^{-6} M IAA. The changes in cell wall deformability were measured using bending methods.[1] The ability of actinomycin D-treated tissue to respond to added auxin through increased cell wall deformability was retained for at least the first hour after auxin addition. However, the actinomycin D apparently reduced the effectiveness of longer auxin treatments. It is as though a small amount of a component essential for cell wall softening, possibly an enzyme, were allowed to act in the presence of auxin. With actinomycin D blocking further synthesis this component would then be depleted during the auxin treatment period.

The following scheme is proposed as an aid to further investigation:

1) Enzymes produced during the course of abscission are capable of cell wall softening. Continued production of these enzymes is essential for abscission to proceed.

2) The cell membrane that normally acts as a barrier restricting the free movement of enzymes must be altered in such a way as to permit entry of wall softening enzymes into the region of the cell wall.

3) In this scheme, auxins could regulate abscission by affecting enzyme production. However, since actinomycin D apparently does not prevent initial functioning of the auxin-induced cell wall softening process, auxin might influence abscission in a manner similar to chloroform, i.e., by affecting the properties of the cell membrane.

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* Morré, D.J., and Petric, P. (Paper in preparation).

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VI. PENNSALT SYNTHESIS PROGRAM

Herbert Q. Smith*

Our presentation this year does not fall neatly into "synthesis" and "screening" portions unless we repeat much of what was said last year, and include synthetic organic chemical details that we feel would be of limited interest to the majority of the group here. We are therefore including structure-activity relationships in both talks, today and tomorrow.

Let me remind you briefly that prior to undertaking work on the Fort Detrick defoliants contract, Pennsalt had specific backgrounds on several defoliant types. We had conducted a fairly substantial program on compounds related to Endothall and a somewhat smaller one on compounds related to butynediol. These were the most significant blocks of data on defoliation per se, but in-house screening programs on herbicidal and other plant response effects provided some other more or less valid leads.

Let me also remind you of the mode of operation of our current synthesis effort. Periodically, candidate structure lists of compounds proposed for synthesis are submitted to Fort Detrick. These lists are checked by Mr. Wiswesser** to avoid possible conflict with the work of other contractors and possible duplication of compounds already tested by Fort Detrick. The selection of compounds to be included on the candidate lists will be discussed later. After approval of the candidate compounds is received, the compounds are assigned to the senior synthesis chemists, working alone or in association with a junior chemist or technician. For maximum efficiency, each synthesis chemist maintains a substantial backlog of assigned compounds and usually works on several in parallel. Considering the various steps the chemist must take on each compound, i.e., checking the literature, deciding on the synthetic approach, ordering starting materials, carrying out the synthesis, isolating and purifying the product, establishing the identity of the product - by suitable physical properties, if known, or by appropriate elementary and spectral analytical data, if novel - and attendant paperwork, the backlog enables these operations to be done in most orderly fashion.

The selection of compounds for inclusion in the candidate lists is based on a number of considerations:

1) Compounds related to known defoliant, desiccant, and herbicidal substances, perhaps even remotely.

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* Pennsalt Chemicals Corporation.
** See page 83.

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2) Compounds that might be considered to be active on the basis of hypothetical biochemical mechanistic considerations.

3) Leads from Pennsalt's own screening results, prior to the government contract work.

4) Novel structures which, to our knowledge, have not been evaluated on biological systems. To this end, close attention is paid to current literature.

5) Feedback of results from our own and Fort Detrick testing.

Suggestions for candidate list compounds are obtained from many sources, including the bench chemists working on the project, the synthesis and screening supervisors, and higher technical management personnel. In fact, the interest in the operation of the project by upper management personnel has been gratifying (and only occasionally disturbing) to us who are working on the project.

The intermediates in the synthesis program are also screened and, when warranted, are included in the candidate lists. Occasionally, unexpected products or by-products are obtained from the synthesis work. These are also tested, provided that their structures can be adequately characterized.

To expand further on the second category listed above, a consideration of the structures of some biologically active compounds has been useful. Since we have not been particularly hampered by a profound understanding of the mechanisms of leaf senescence and abscission, it has appeared a valid approach to explore some model compounds of types known to have biological activity, not necessarily limited to phytotoxic or plant growth regulator effects. Thus, some known metabolic inhibitors and appropriate derivatives have been selected for synthesis and screening. In only a few cases has any sort of antimetabolite activity been displayed. We have, on t   other hand, laid considerable emphasis on potentially reactive compounds that might be expected to have some biological action by "alkylation" of sites essential to enzyme activity.

In retrospect we can say that, given an active prototype compound, we may make some or all of the following modifications:

First, we may alter an alkyl substituent to change the hydrophilic-hydrophobic balance. Not infrequently we have included methyl, ethyl, butyl, and octadecyl homologs. If the level of interest continues high we may also include octyl, dodecyl, and benzyl. I think that in only two cases, however, has this entire series been surveyed. If there is reason to suspect that steric hindrance plays a role or if the alkyl group is attached to oxygen, nitrogen, or sulfur, and hydrolysis may be a factor in the compound's activity, we may compare primary, secondary, and tertiary alkyl groups. We may also look at saturated and unsaturated alkyl substituents. Alkyl and aryl substituents are, of course, frequently compared.

Second, an oxidizeable function may be introduced in several stages of oxidation. For example, we may compare a primary alcohol, its aldehyde, and its carboxylic acid; a sulfhydryl group with its disulfide, its sulfinic acid and its sulfonic acid; a sulfide with its sulfoxide and sulfone.

Third, in many cases we have compared oxygen and sulfur analogs and occasionally introduced selenium as well.

Fourth, substituents may be introduced on the basis that they may be expected to alter the electronic configuration of the functional groups of the molecule that are presumed responsible, or at least highly involved, in the biological activity.

Fifth, wherever we are dealing with a bifunctional molecule, such as butynediol or Endothall, we may alter the distances between the functional sites. In the case of the two compounds just mentioned and with diquaternaries, these distances may be quite critical. As you are aware, there are three stereoisomers of Endothall, exo-cis, endo-cis and trans. The first of these is highly active, the second much less so, the third practically inactive. Obviously, spatial relationships are important.

Sixth, where steric hindrance appears to be a factor in promoting or limiting activity, we may remove or introduce blocking groups.

Seventh, if the compound is ionic and it appears likely to be blocked from ready entry into the leaf or tied up in such a way as to limit translocation, we may prepare a nonionic derivative, for example, the conversion of an acid to a fragile ester.

Many other planned modifications suggest themselves and, if one could have no feedback of data on plant responses beyond information on the activity of the prototype compound, a program planner would have to rely heavily on such a systematic approach. However, with the rapid feedback of our own and Fort Detrick data, we can follow our structural leads quite promptly with the result that we can be somewhat less routine and thereby more productive.

The proposal for our first contract, which was presented something over two years ago, outlined work which we felt was warranted to further explore those defoliant types for which some background was available to us. In addition we suggested the exploration of a rather wide variety of chemical structures. For the purpose of meaningful reporting we have classified and reclassified so that the structural categories that we now use are no longer identical with those originally proposed. Some categories have been dropped as unpromising, some have been added. The following principal categories are now under investigation:

Endothalls and other bridged compounds
Unsaturated compounds
Heterocyclic compounds
Sulfur compounds
Carbamates
Ureas
Thiocyanates and isothiocyanates
Phosphorus compounds
Phenols and derivatives
Carboxylic acids
Miscellaneous

You will note, of course, that some of these are exceedingly broad; nevertheless they have served a useful purpose in the organization of monthly screening reports and quarterly reports.

Naturally, one of the first areas in which we carried out extensive work was Endothall. Figure 1 summarizes the structure activity relationships established thus far.

We can go into further detail in the case of the Endothallamic acids as shown in Figure 2.

A generalized conclusion appears to be that the activity of Endothall derivatives may be related to the ability of the compound to revert to Endothall after application. However, there is much evidence of species selectivity, especially with the Endothallamic acids, perhaps related to absorption, penetrability, etc. The synthetic effort in this area has been substantially reduced, pending receipt of further information on this last point from tests on woody plants.

Figure 3 indicates some work which was carried out to prepare variations of butynediol. Structures in which one hydroxyl group was replaced by a number of different substituents are shown with their activities on beans at 1 lb per acre. As a rough conclusion, compounds which can revert to butynediol in the plant retain or exceed the activity of the parent compound. Bis derivatives of butynediol are generally inactive, including amines, ethers, esters, acetals, etc., except where incorporating an active moiety, such as Endothall.

The next type of structure resulted from some work done early in the program in an attempt to improve upon the known herbicidal activity of ammonium thiocyanate, without notable success. Several higher alkyl isothiocyanates were prepared, because of the possible relationship to the thiocyanates and because they were reasonably reactive. Figure 4 gives the results obtained with a series of these compounds. Maximum activity appears to reside in straight chain, saturated, molecules equivalent in length to fatty acids.

X	Y	Z	Activity (beans)
$\overset{O}{\underset{\|}{-C}}-N(A)$	$\overset{O}{\underset{\|}{-C}}-$	-	Nearly all inactive
$-CO_2H$	$-CO_2H$	$4,5-Cl_2$; $4,5-Br_2$; $1,2-Me_2$; OH, F,. $-OAc$, $-SO_3Na$, unsaturation, etc.	Inactive
$-CO_2M$	$-CO_2M$	-	Retain much of activity
$-CO_2R$	$-CO_2H$	-	Some activity
$-CO_2R$	$-CO_2R$	-	Less than monoesters
$-CN$	$-CO_2R$	-	Much reduced activity
$-CN$	$-CN$		
$-CO_2H$	H	-	Inactive
$-CH_2-N(A)-CH_2-$		-	
$-CH_2-N(A)-\overset{O}{\underset{\|}{C}}-$		-	Inactive
$-CH_2OA$	$-CH_2OB$	-	

Replacement of O by $(CH_2)_n$ where n = 1 or 2, inactive

Figure 1. Endothall Structure - Activity Summary.
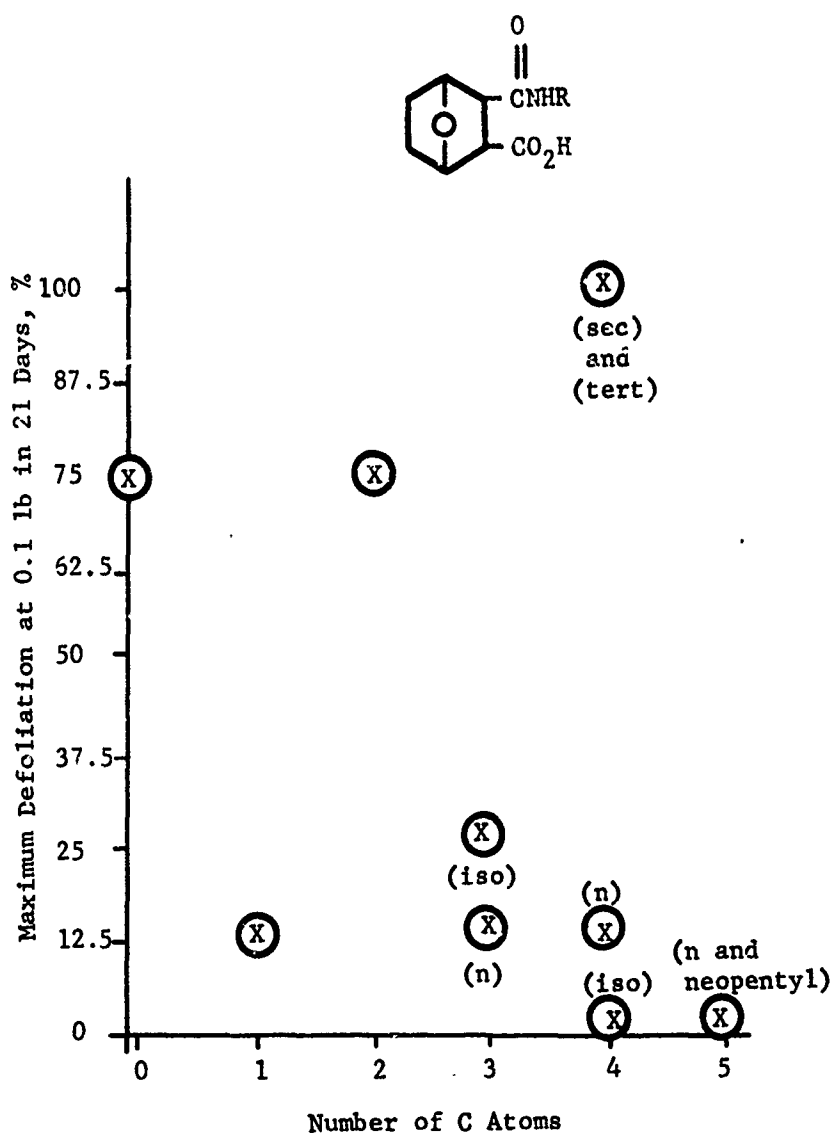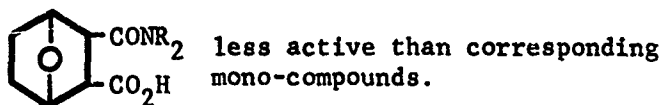
Figure 2. Activities of Various Endothallamic Acids

$$HOCH_2C{\equiv}CCH_2X$$

X	Def. at 1 lb (beans)
HO	Yes
H	No
Cl	Yes
Br	Yes
I	Yes
CH_3-	No
C_2H_5-	No
$KOSO_2O-$	No
$(C_2H_5)_2N-$	No

Figure 3. Defoliation Activity Displayed
by Some Acetylenic Compounds.

Even a superficial review of the patent literature indicates that a variety of organic structures has been shown to exhibit phytotoxic effects of one sort or another. We have considered it neither feasible nor desirable to avoid compound types revealed in the patent literature even though it was recognized that situations might arise in which it would be impossible for us, as the contractor, to assure the government of royalty rights not dominated by an existing patent.

For example, an initial look at several substituted ureas suggested that some compounds in this category were more effective defoliants in the primary screening on beans than known urea herbicides. Thus, some work in this much-patented area was indicated.

Removal of one methyl group from the commercial herbicide, fenuron, gave a compound which was more active on bean plants. However, moving one methyl group of fenuron to the other nitrogen atom gave a much less active compound. These relationships did not necessarily apply when N,N-dialkyl-N'-2-benzothiazolylureas, N,N-dialkyl-N'-(1-indanyl)ureas, or N-aryl-N,N'-(alkyl-alkynyl)ureas were employed.

In our next presentation, we will discuss structure-activity relationships of still more structural types as well as details of our screening work.

R-NCS

$\underline{R}$	$\underline{\text{Def. at}}$
C_4H_9-	0
C_8H_{17}	10 lb
tert-$C_8H_{17}-$	0
$C_{11}H_{23}-$	10 lb
$C_{12}H_{25}-$	1 lb
tert-$C_{12}H_{25}$	0
$C_{16}H_{33}-$	1 lb
$C_{15-18}H_{33-37}-$(from soyamine)	1 lb
$C_{16-18}H_{33-37}-$(from tallowamine)	10 lb
$C_{18}H_{37}-$	0.1 lb
tert-$C_{19-23}H_{37-45}-$	10 lb
$C_6H_5CH_2CH_2-$	10 lb

Figure 4. Defoliation Activity of Some Isothio-cyanate Compounds.

VII. ETHYL DEFOLIANT SYNTHESIS PROGRAM

John C. Wollensak*

In describing the synthesis portion of the defoliant contract work at the Ethyl Corporation, we will discuss the organization of our program and the manner in which compounds are selected and then synthesized. We also will look at some of the classes of compounds that have been screened in the program and the sources of these materials. In the next portion of this paper, we will consider specific chemical structures and activity relationships. The formal organization of our present program is shown in Figure 1. Dr. Rifkin is the project director. Professor Hall of Texas A and M University and Dr. McNew, Director of Boyce Thompson Institute, are consultants for the defoliant program. Dr. Plaisted of Boyce Thompson Institute directs the screening operations for the program; Dr. Closson supervises the synthesis program.

The synthesis chemist's first, and perhaps most important, task in a program such as this is to select compounds to be synthesized. The compounds are selected on the basis of screening results, pertinent literature, and new or established theories of herbicide or defoliant activity.

Screening results are important because they permit correlation of activity with the structure of compounds and with such properties as solubility, polarity, stability, and volatility. The structures of active molecules can be modified in future synthesis work to enhance the herbicidal activity or to reduce mammalian toxicity and other undesirable properties. After the program has become established, the screening results become the most important basis for the selection of compounds.

Also important is the extensive literature on herbicidal compounds. By indicating the areas of activity previously uncovered, it provides one starting point for our synthesis program. On the other hand, the literature sometimes indicates compound classes that are inactive. Probably most helpful to the program are the published herbicidal activities of series of compounds. These often indicate the effect of functional groups on activity — information that can be transferred to other chemical classes.

An alternate approach to the choice of compounds makes use of proposed mechanisms of herbicidal action. For example, preparation of materials with the proper balance between lipophilic and hydrophilic characteristics allows them to penetrate the leaf membrane and to be translocated in the plant. Metal chelating properties or oxidizing properties of compounds

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* Ethyl Corporation.
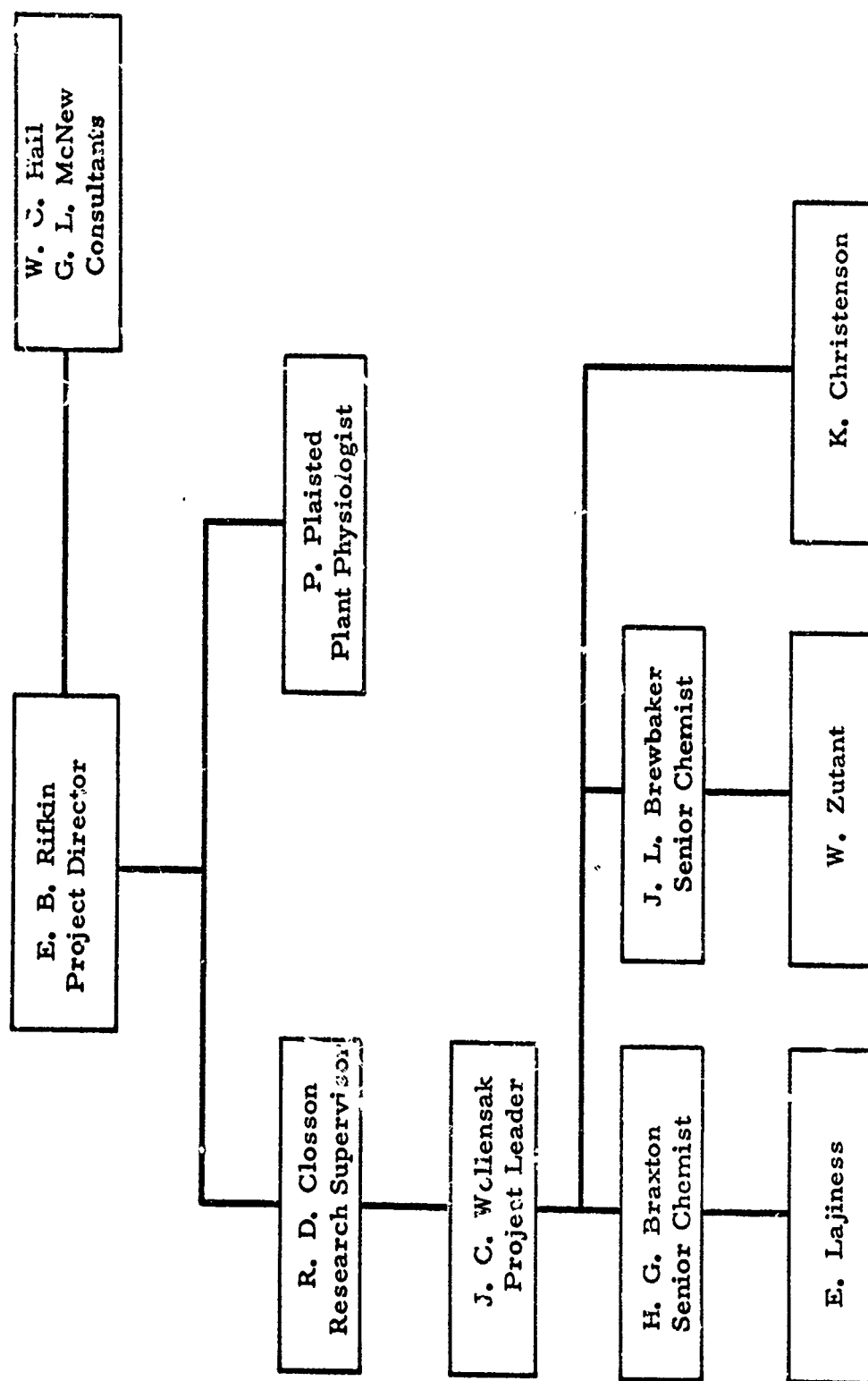
Figure 1. Defoliant Program Organization.

may inactivate enzyme sites causing the desired effect. Attempts have been made to prepare compounds that produce low-molecular-weight defoliants, such as ethylene directly in the plant. Hypotheses of this type can be helpful as a basis for the selection of compounds.

After compounds are selected, sources for obtaining the material may be:

1) Synthesized for the program and intermediates prepared during multi-step syntheses,

2) Largely of the organophosphorus type, available from other laboratory programs, and

3) From commercial sources and other chemical laboratories.

The best synthetic approach is chosen for those materials that cannot be procured from external sources. In most instances, standard synthetic methods can be used to synthesize the desired compounds. Thus, we need not discuss at length the synthesis of individual compounds.

During discussion of the screening results, we will list individual chemical structures and their screening data. Compounds previously described in the literature are characterized by their physical properties and by instrumental analysis. New materials are also characterized by elemental analysis and, occasionally, by proof of chemical structure. In multi-step syntheses, all intermediates are also submitted to the screening program. We thus examine all materials prepared, even though the probability of finding high activity in some of the intermediates is quite low.

In addition to compounds synthesized for the program, we have made extensive use of chemicals available from other laboratories, from some of the other programs at Ethyl, and from commercial sources. By using materials from these alternate sources, we can test hypotheses of plant-response activity at lower cost than via the corresponding syntheses. With these materials, we can introduce new and different types of structures into the program, thereby uncovering leads and opening new areas of investigation. Where activity has already been established, these other sources of materials may fill out the active area or, on occasion, provide an herbicide with very high activity. Generally speaking, we have found these compounds from other sources to be very helpful in the conduct of the program.

Some of the classes of compounds that have been screened in the defoliant program are as follows:

Acetylenes	Carboxylic Acid Esters
Amides and Thioamides	Diazines
Amines and Amine Oxides	Dioxalanes and Sulfur Analogues
Biuret Salts	Disulfides
Carbamates and Thiocarbamates	Halogen Compounds
Organogermanium Compounds	Thiazines
Organolead Compounds	Thiazoles
Organomercury Compounds	Thiocarbonates
Organophosphorus Compounds	Thiols
Organotin Compounds	Thiopseudoureas
Sulfides, Sulfoxides and Sulfones	Triazines
Sulfonates	Ureas and Thioureas
Thiadiazoles	Miscellaneous

Neither the synthesis effort nor the evaluation of compounds was distributed evenly among these classes. A few compound types, such as acetylenes, organometallics, heterocyclics, and organophosphorus compounds have received a large share of our effort. Organometallics, like the other areas of major concentration, were originally selected for a number of reasons. It has been known for some years that certain organometallic classes, especially those containing tin and mercury, affect plant growth. Some of the personnel on the project have had considerable experience in the field of organometallic synthesis. In addition, a number of organometallic compounds were available for screening from other company synthesis programs and from other laboratories. Synthesis of the organometallic compounds of tin and lead was conducted, for the most part according to published procedures. We have prepared the mono-, di-, tri-, and tetraalkyltin compounds with selected alkyl chain lengths ranging from C_1 to C_{12}. Aryltins, aralkyltins, and alkenyltin compounds have also been screened.

During the first year, little synthesis of organolead compounds was carried out. However, screening data were obtained in this area on compounds obtained from other programs. Likewise, in the organomercury area, many compounds were obtained from proprietary programs and from external sources. These materials were supplemented by a small synthesis effort. One of the chemical reactions used in preparing organomercurics was the well-established addition of mercuric salts to olefins. Eighteen mercury compounds were screened during the first year of the program. Synthesis of a number of boron and silicon compounds was directed toward a particular mode of activity in or on the plant. Of the organic compounds of the 26 metallic elements screened, those of mercury, tin, germanium, and lead have been the most active.

The large effort on organophosphorus compounds was a result of two factors: (i) they have been recognized for some time as herbicidal materials; and (ii) we had a number of these compounds on hand at the start of the program. Moreover, we wanted to pursue leads uncovered in an agricultural-chemical program conducted at Ethyl Corporation from 1947 to 1954. In that program, a number of phosphorus compounds were found to have high activity, particularly in the areas of defoliation and other plant-growth effects. The discoveries of that agricultural-chemical program were eventually sold to Chemagro, and one of the chemicals discovered is "DEF" (S,S,S-tri-n-butyl phosphorotrithioate).

Other organophosphorus leads uncovered in that program were pursued during the first year of the defoliant contract, and study of many new phosphorus areas has been initiated. In the phosphorus area as well as with organometallics we use established reactions extensively. We have used the Arbuzov preparation of phosphonates and other phosphorus compounds as well as various esterification and amidation reactions. Organometallic reagents such as the Grignards are used in preparation of phosphines, phosphine oxides, and related compounds.

More than 200 phosphorus compounds were submitted for primary evaluation during the first year of the contract, a large number of which were available in the laboratories when the program was started. We have evaluated phosphine oxides, phosphinates, phosphonates, and phosphates, plus most of the nitrogen and sulfur analogs of these compounds. Compounds with phosphorous of valence three and the phosphonium compounds have also been examined to some extent.

In some of the other areas such as acetylenes and heterocycles, activity observed during the first year of the program was not as high as in the organometallic area. However, we did find some moderately herbicidal materials that are useful as leads for further synthesis.

In the section on screening data, we will specifically discuss the most active compounds, the primary and secondary screening data, and the compounds selected for field testing.

VIII.   MONSANTO SYNTHESIS PROGRAM

Stanley D. Koch*

For more than a year and a half Monsanto Research Corporation has conducted one of several synthesis and screening programs with the Crops Division.   Monsanto Research Corporation is the research arm of Monsanto with responsibility for conducting Government contract research.

The synthesis of compounds in our defoliant program has been carried out largely at our Boston laboratory; up to 25% of the compounds are prepared at our Dayton, Ohio, laboratory. The synthesized materials are sent for screening to the Crops Division, and also to our own screening program, the Agricultural Research Laboratory in St. Louis. The selection of compounds to be synthesized and tested comes both from the chemists and biochemists in Boston and Dayton and from the plant physiologists and agronomists in St. Louis.

The organization of the program at the three locations is as follows:

Dr. Stanley D. Koch
Project Leader

SYNTHESIS, MONSANTO RESEARCH CORPORATION		SCREENING, MONSANTO COMPANY Agricultural Division
Dr. Stanley D. Koch Synthesis, Boston	Dr. William H. Yanko Synthesis, Dayton	Dr. Philip C. Hamm Screening, St. Louis

In searching for ideas for new compounds to be evaluated as defoliants, we have been looking for a meaningful breakthrough. We have felt that development of a material that is only a few per cent more effective than known commercial products would be of no real value. For this reason, with one exception to be mentioned later, we have avoided suggesting compounds that were merely analogs of known herbicides or defoliants as such analogs have usually been widely tested for herbicidal applications.

For this reason, a portion of our suggested compounds has always been the exotic, out-of-the-way organic compound. We recognize, as well as anyone with experience in biological screening, that the shot-in-the-dark approach faces prohibitive odds. Therefore we require a compound to be more than merely exotic. There must also be at least the shadow of a rationale. It must be possible to relate the chemical functionality of the candidate compound to some class of compounds with known biological

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* Monsanto Research Corporation.

activity, preferably against plants. During the past year this has been further circumscribed by the elimination of classes that were insufficient y differentiated from classes shown to be inactive during our first year of screening.

Last year at this conference I called this approach the "rational screen." So far, the rational screen has given us three active classes of compounds: alkyl isothiocyanates (which might hydrolyze in the field to thioureas), aryl boronic acids, and a totally unexpected group of phosphinate esters, often halogen substituted. Examples from these classes are:

1) Isothiocyanates $C_6H_5CH_2CH_2-NCS$

2) Boronic acids $Br-\langle\;\rangle-B\begin{smallmatrix}OH\\OH\end{smallmatrix}$

3) Phosphinates $(C_4H_9)_2-\overset{\downarrow}{\underset{O}{P}}-O-\langle\;\rangle-CF_3$
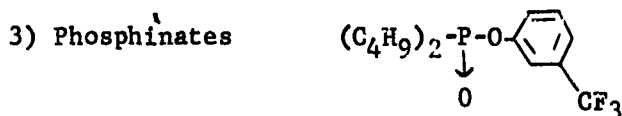
Besides the rational screen, another source of leads are those arising from the years of agricultural chemical research done by Monsanto Company. Our most active class against woody species is in this class: the quaternary ammonium iodides.

REPRESENTATIVE ACTIVE QUATERNARY AMMONIUM IODIDES

$H_3\overset{+}{N}-CH_2CH_2-\overset{+}{N}H_3 \quad 2I^-$

$CH_3-\langle\;\rangle-\overset{+}{N}-CH_3 \quad I^-$

$\left[\begin{smallmatrix}O\\N-CH_2CH_2CH_2-N(CH_3)_3\\O\end{smallmatrix}\right]^+ \quad I^-$

$(CH_3)_4\overset{+}{N} \quad I_5^-$
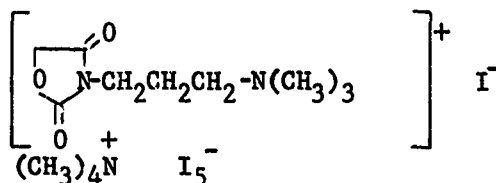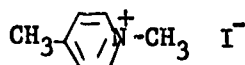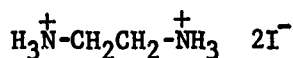
In this class are compounds that defoliate several species of trees (70% in as few as two days), compounds that show 50% defoliation in 14 days at 5 pounds per acre against as many as five of our seven woody species, and compounds that show 50% defoliation in 14 days at even 1 pound per acre against two of the tree species.

Ironically, these iodides are not particularly active against several of the species in the Fort Detrick primary screen.

Another active group are the other organic iodides.

OTHER IODINE-CONTAINING COMPOUNDS

CH_2I

$HO-\langle\overline{\bigcirc}\rangle-NO_2$

$CH_3S-\langle\overline{\bigcirc}\rangle-O+\ \ I^-$ with CH_3 (top) and CH_3 (bottom)

$C_6H_5-NHCOOCH_2CH_2I$

$CH_3(CH_2)_{17}-\overset{+}{\underset{CH_3}{S}}-C_2H_5\ \ \ I^-$

$(C_2H_5)_4\overset{+}{P}\ \ I^-$
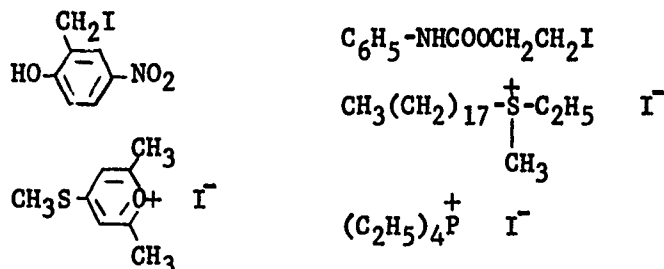
Because of the known instability of organic iodides in the presence of air and ultraviolet light, it is quite possible that even covalent iodine compounds act by producing iodide ion.

The last major source of our leads are the less well-known herbicides. Here we find the organotins, a class known to have general biological activity. We have found in this group some very active compounds active both against trees and grasses. Examples are:

REPRESENTATIVE ACTIVE TIN COMPOUNDS

$$(CH_3)_3-Sn-O\overset{\overset{O}{\|}}{C}-CH_2CN$$

$(CH_3)_3-SN-CN$

$(C_4H_9)_3-Sn-O-\langle\overline{\bigcirc}\rangle-Cl$ with Cl
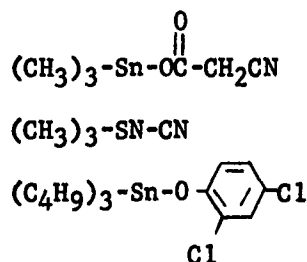
Another class based on less well-known herbicides is a group of halogen-containing organics related to some of Monsanto's active herbicides. These are sometimes quite active as defoliants. A final example is a group of compounds related to a halophenol herbicide reported in the open literature only in 1963, and still rather obscure. These compounds are especially potent desiccants.

Our newest leads could be said to be the last-mentioned group of halo-phenols, compounds such as our D35273 and C35683; and a group of phosphorus compounds (A36838, A36842, A36848, A36840, and A36846). This group does turn out to be a specific modification of a known defoliant but shows signs of having better defoliant activity than the parent.

Synthesis of the latter compounds is now complete and the crucial screening tests should be completed in the next month or so.

Our method of operation is as follows: compounds are suggested for synthesis by any of the project staff at Boston, Dayton, or St. Louis. In several cases chemists at Boston assigned to other programs have come forward with suggestions and some of these have proved to be active. At monthly intervals these suggestions are gathered, edited, and sent to Crops Division for approval for synthesis. At the same time, the same list is submitted to the parent company's Sample Record and Control Office in St. Louis. This is the nerve center of Monsanto's computerized index of chemical compounds and testing results. Clerks at Sample Record and Control office notify us if the candidate compounds have ever been screened for defoliant and herbicidal action by Monsanto Company. If they have, some data on activity are known and they are not rescreened at Government expense unless remarkably active. After Crops Division and Monsanto's Sample Record and Control have both informed us that the candidate compounds have not previously been screened, they are available for synthesis. Compounds suggested in Boston and St. Louis are prepared in Boston, and those suggested in Dayton are prepared there.

The highest standards of preparation and purity are maintained. No compound is submitted whose identity has not been verified. Purity is almost always more than 95%, usually much higher, and verified by method of preparation, infrared spectroscopy, vapor-phase chromatography, nuclear magnetic resonance, or other appropriate means. In general, the standards for work reportable to the Journal of Organic Chemistry are adhered to.

Samples for screening are shipped weekly to Fort Detrick and to the screening laboratory. Our own screening will be described by Dr. Hamm.

Feedback of results from both of these screens serves to modify our synthesis in two ways. Leads are followed up, and inactive classes are abandoned after sufficient evidence of their inactivity has accumulated. The latter always involves some danger of missing a good lead. There are many examples in biological action of chemicals where an active compound is surrounded by absolutely inactive homologs.

About 15% of the compounds submitted have not been synthesized, but purchased. We have done this without apology: these compounds have been analogs of leads (or the leads themselves). A larger percentage of the purchased compounds than of the synthesized compounds have been found to be active against the woody species, showing only that the former represent feedback information to a greater degree than do the synthesized compounds.

About a quarter of the submitted compounds represent exploitation of our most active groups, or of known herbicides. Of course, if presently contemplated compounds were included as well as those sent out, the ratio would be appreciably higher.

This completes my description of our method of choosing compounds for synthesis and the mechanics of our synthesis program. In his talk, Dr. Philip C. Hamm, of Monsanto's Agricultural Division, will describe our screening program and some of the results.

IX. PREPARATION OF NEW ARSINIC ACIDS

Dr. E.O. Leonard*

General Aniline has been screening new organic compounds for use as herbicides for a number of years. Our interest in the present contract work is twofold. First, we had found a number of biologically active groups in the course of our herbicidal screening, and secondly, we have in the company a man with an unusual background of experience in arsenic chemistry, Dr. J.F. Morgan.

Figure 1 shows two very effective defoliants, cacodylic acid (dimethyl arsinic acid), and butynediol.

Cacodylic Acid   **Butynediol**

$$CH_3 \diagdown \quad \underset{\overset{\|}{As}}{O} \!-\! OH$$
$$CH_3 \diagup$$

$HOCH_2C\equiv CCH_2OH$

Proposed

$$R' \diagdown \quad \overset{O}{As} \!-\! OH$$
$$R \diagup$$
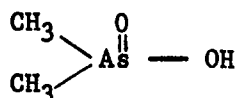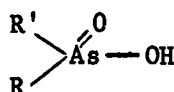
Figure 1. Preparation of New Arsinic Acids and Esters

In our contract work, we proposed to vary the alkyl groups of the arsinic acids through the incorporation of biologically active groups, particularly those containing triple bonds and related to butynediol.

The methods for the preparation of alkyl arsinic acids and their intermediates are shown in Figure 2. In the first step, sodium arsenite is treated with an alkyl halide, preferably a bromide, to produce an alkyl arsonic acid salt. This is known as the Meyer Reaction. In the second step, the salt is reduced with sulfur dioxide in concentrated hydrochloric acid solution to produce an alkyl dichloroarsine. This intermediate is then converted with caustic to the disodium alkyl arsonite. If this compound is alkylated with another alkyl halide a dialkyl arsinic acid will be produced. This latter reaction is an extension of the Meyer Reaction.

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* General Aniline & Film Corporation.

Alkyl Intermediates

1) $As(ONa)_3 + RX \longrightarrow RAsO(ONa)_2$ (Meyer Reaction)

2) $RAsO(ONa)_2 + HCl + SO_2 \longrightarrow RAsCl_2$

3) $RAsCl_2 + NaOH \longrightarrow RAs(ONa)_2$

Alkyl Arsinic Acids

4) $RAs(ONa)_2 + R'X \longrightarrow$ R' $\diagdown$ AsO_2Na (Meyer Reaction)
                                        R $\diagup$
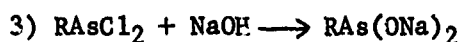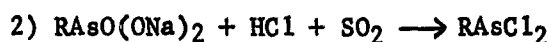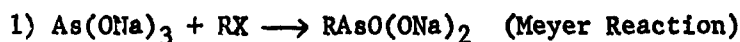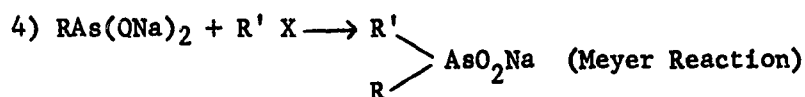
Figure 2.   Preparation of Arsinic Acids.

Figure 3 shows several dialkyl arsinic acids synthesized by the method
just described.   Allylpropylarsinic acid was prepared by the reaction of
allyl bromide on disodium propyl arsonite.   2-Hydroxyethylbutyl arsinic
acid was prepared by the reaction of ethylene oxide on disodium butyl
arsonite, and the third compound shown was prepared by the reaction of
ethylenechlorobromide on disodium propyl arsonite.   This latter compound,
ethylenebispropylarsinic acid, was isolated from a reaction solution
which was intended to produce 2-chloroethylpropylarsinic acid.   Attempts
to produce several 2-chloroethylalkylarsinic acids by varying reaction
conditions have been unsuccessful to date.

$$H_2C{=}CHCH_2-\overset{\overset{\displaystyle O}{\|}}{As}-CH_2CH_2CH_3$$
$$\underset{OH}{|}$$

$$HOCH_2CH_2-\overset{\overset{\displaystyle O}{\|}}{As}-CH_2CH_2CH_2CH_3$$
$$\underset{OH}{|}$$

Allylpropylarsinic Acid   2-Hydroxyethylbutylarsinic Acid

$$CH_3CH_2CH_2-\overset{\overset{\displaystyle O}{\|}}{As}-CH_2CH_2-\overset{\overset{\displaystyle O}{\|}}{As}-CH_2CH_2CH_3$$
$$\underset{OH}{|} \qquad \underset{OH}{|}$$

Ethylenebispropylarsinic Acid

Figure 3.   Dialkylarsinic Acids.

The methods used to prepare aryl arsinic acids are shown in Figure 4. In the first reaction, sodium arsenite is treated with benzene diazonium chloride to produce a phenyl arsonic acid salt by the Bart Reaction. This can then be reduced in the same way as the alkyl derivatives to form phenyl dichloroarsine. Reaction of the phenyl dichloroarsine with alkali yields disodium phenyl arsonite. Aryl arsinic acids can be prepared by either of two methods. Reaction 4 shows the formation of an aryl alkyl arsinic acid. The disodium phenyl arsonite was prepared by the Bart Reaction, and the Meyer Reaction was used to prepare the final arsinic acid product. Reaction 5 shows the reverse. The Meyer Reaction was used to prepare the disodium alkyl arsonite and the final product synthesized by the Bart Reaction. Arsinic acids containing nitrophenyl groups must be prepared by the method shown in Reaction 5 because the nitro groups would be reduced to an azoxy group by the trivalent arsenic formed in Reaction 2.

Aryl Intermediates

1) $As(ONa)_3 + C_6H_5N_2Cl \longrightarrow C_6H_5AsO(ONa)_2$ (Bart Reaction)

2) $C_6H_5AsO(ONa)_2 + HCl + SO_2 \longrightarrow C_6H_5AsCl_2$

3) $C_6H_5AsCl_2 + NaOH \longrightarrow C_6H_5As(ONa)_2$

Aryl Arsinic Acids

4) $C_6H_5As(ONa)_2 + RX \longrightarrow C_6H_5(R)AsO_2Na$

5) $RAs(ONa)_2 + O_2N-C_6H_4N_2Cl \longrightarrow O_2N-C_6H_4-\overset{\displaystyle |}{\underset{\displaystyle R}{As}}O_2Na$ (Bart Reaction)

Figure 4. Preparation of Arsinic Acids.

Figure 5 shows several alkylarylarsinic acids prepared as shown in Figure 4. The first two compounds, allylphenylarsinic acid and allyl-o-chlorophenylarsinic acid were prepared by the synthesis of the aryl intermediate followed by alkylation with allyl bromide as in the Meyer Reaction. The third compound, ethyl-p-nitrophenylarsinic acid was synthesized by the reaction of p-nitrophenyldiazonium chloride on the ethyl arsonite intermediate as in the Bart Reaction.

$$H_2C{=}CH{-}CH_2{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{As}}{-}\langle\text{phenyl}\rangle$$

Allylphenylarsinic Acid

$$H_2C{=}CHCH_2{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{As}}{-}\langle\text{Cl-phenyl}\rangle$$

Allyl-o-Chlorophenylarsinic Acid

$$CH_3CH_2{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{As}}{-}\langle\text{phenyl}\rangle{-}NO_2$$

Ethyl-p-Nitrophenylarsinic Acid

Figure 5. Alkyl Aryl Arsinic Acids.

In Figure 6 are shown several compounds prepared by the reaction of aryl arsonites with epoxides. The aryl intermediates were prepared by the Bart Reaction while the final arsinic acid products were synthesized by a method analogous to the Meyer Reaction. Maximum yields were obtained by carrying the reactions out at room temperature and using an excess of the epoxides. Several reactions at elevated temperatures (60 to 100 C) resulted in the formation of higher concentrations of glycols.

$$R{-}\overset{\overset{\displaystyle ONa}{\diagup}}{As}{-}ONa + R'{-}\overset{\overset{\displaystyle O}{\diagdown}}{CH}{-}CH_2 \xrightarrow[25\ C]{NaOH} R{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{As}}{-}CH_2{-}\overset{\overset{\displaystyle OH}{|}}{CH}{-}R'$$

R	**R'**
Phenyl	Methyl, Ethyl, Phenyl
o-Chlorophenyl	Methyl
m-Chlorophenyl	Methyl, Ethyl, Phenyl
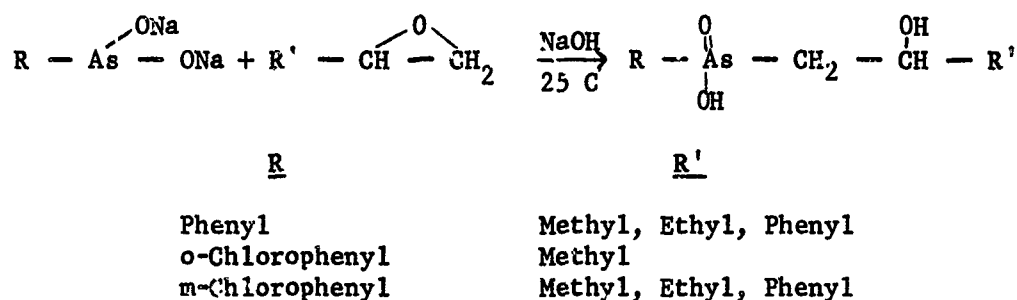
Figure 6. Alkyl Aryl Arsinic Acids Prepared by the Reaction of Aryl Arsonites with Epoxides.

Figure 7 shows the reaction of cyclohexene oxide with sodium phenyl arsonite or sodium m-chlorophenylarsonite.

$$\underset{\displaystyle\text{ONa}}{R - \overset{\displaystyle\text{ONa}}{\underset{\displaystyle\text{ }}{As}}} - \text{ONa} + \bigcirc\hspace{-1.4em}^{O} \quad \xrightarrow[\text{25 C}]{\text{NaOH}} \quad R - \overset{\displaystyle\text{O}}{\underset{\displaystyle\text{OH}}{\overset{\displaystyle\parallel}{As}}}\hspace{-0.3em}\cdot\hspace{-0.3em}\overset{\text{OH}}{\bigcirc}$$
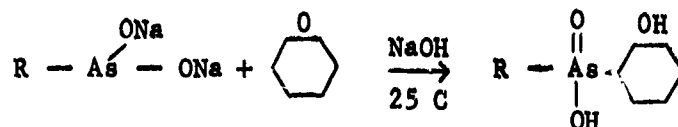
R = Phenyl, m-Chlorophenyl

Figure 7. Reaction of Cyclohexane Oxide with Sodium Phenyl
            Arsonite or m-Chlorophenylarsonite.

In Figure 8, the methods of isolating the final products are shown.
The standard procedure of concentrations, filtrations, and gradual acidi-
fication of the reaction filtrate, was used on all reactions. In many
cases, especially the reaction of epoxides, two or three layers were
present. The product could be isolated from either of the layers, depend-
ing upon the particular reaction. The third layer, not shown on the slide,
and if present, was a layer of solid usually suspended between the other
two layers. The solids were separated by filtration and dissolved in
water. Acidification of this solution usually resulted in isolation of
the product.

1) Concentration and Gradual Acidification to pH 3

    a. Reaction Filtrate
    b. Aqueous Layer
    c. Organic Layer

2) Ion Exchange Resin (For Removal of NaCl)

Figure 8. Methods of Isolating Final Products.

The second method of isolation involved the use of the acid form of
sulfonic acid ion-exchange resins. These were used primarily to remove
sodium chloride from some of the more water-soluble arsinic acid products.
The HCl was removed by eluting with water. The arsinic acids were then
eluted with more water. In some cases it was necessary to elute with 0.1 N
NH_4OH solution. The product came off the column just before the NH_4OH.

In Figure 9 are shown two methods used to identify the products. A
simple titration of a purified product with standard alkali will produce
a curve characteristic of an arsinic acid as well as provide an acid
number. Purification of samples by crystallizations can be followed by
quantitative titrations. The curve on the left is typical of arsinic
acids and is readily distinguishable from arsonic acids (middle curve).
Sodium arsenite was titrated with standard acid solution and is presented
here for comparative purposes. It is easily distinguished from an arsinic
acid. The final step in product identification is a quantitative assay
for arsinic. The method used involves digestion of the compound in con-
centrated H_2SO_4. The final analytical sample is titrated with 0.1 N I_2
solution and the per cent arsinic calculated.

1) Titration Curves

Allylpropylarsinic Acid	Benzenearsonic Acid	Sodium Arsenite
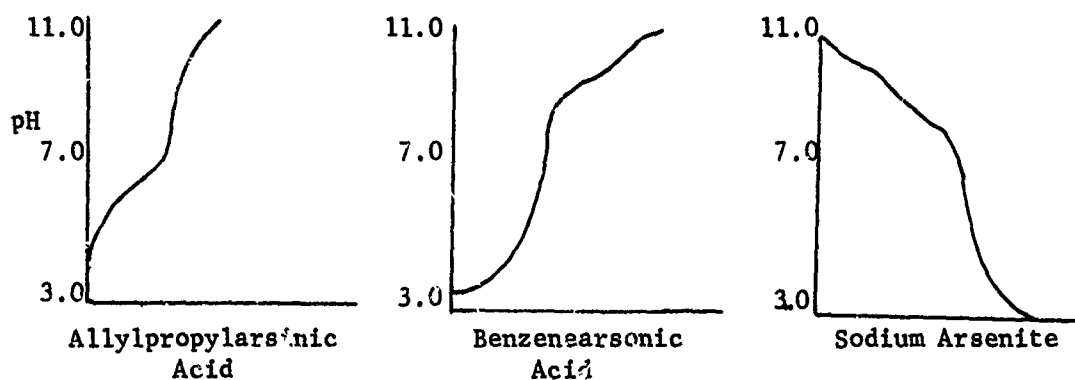
2) Arsenic Assay (H_2SO_4 Deccmposition)

Figure 9. Identification of Arsinic Compounds.

Figure 10 shows some compounds to be prepared. In addition, if contract
time permits, several methoxyphenyl derivatives will be synthesized.

$$R - As \begin{array}{c} ONa \\ \end{array} - ONa + R' - O - CH_2CHCH_2 \xrightarrow[25\ C]{NaOH} R - \overset{O}{\underset{OH}{\overset{\|}{As}}} - CH_2CHCH_2 - O - R'$$

R	R'
Phenyl	Allyl, Propyl, 2,4-Dichlorophenyl
o-Chlorophenyl	Allyl, Propyl, 2,4-Dichlorophenyl

Figure 10. Compounds to be Prepared.

X.   CODING AND DATA PROCESSING OF CHEMICAL INFORMATION

William J. Wiswesser*

This Defoliation Program represents a tremendous coordinated investment in technological information and man-hours of synthesis or screening work, and w nave a major obligation to correlate the resulting multitudes of data efficiently and profitably. In this report we will briefly describe two of our methods for managing the chemical information with the UNIVAC computer equipment in the Biomathematics Division. The first organizes the indexes with fast-searching multidimensional structure measures that we identify as "BATCH" numbers; the second method uses an ingenious "DOT-PLOT" program (written by a member of the Biomathematics Division) to print chemical structure diagrams or tabulations of related screening results at remarkably high speed — potentially some 15,000 diagrams or tables per hour. A complementing computer program will consolidate the crops-screening data in an unwieldy file of some 100,000 cards and make this information more readable and more manageable with about a tenth this number of sorting cards.

A.   CHEMICAL "B-A-T-C-H" NUMBERS

These five decimal digits are designed to decimate large chemical decks by a corresponding number of substantially independent variables: the "benzene ring" or B-number, the "atomic class" or A-number, the "total heteratom count" or T-number, the "carbon-units" count or C-number, and the "summed hydrogen-digit" count or H-number. BATCH-numbers were first described in 1963** at the American Chemical Society Meeting in Chicago. As then explained, most structural searches can be focused to an astonishing degree with just a few natural formula-indexing measures of small magnitude (less than ten) that are easily learned, quickly counted, and always remembered. The BATCH-numbers are five such natural and simple measures.

Figure 1 illustrates the traditional starting point for the logic of BATCH-numbers. These distribution curves show how a large chemical file is very poorly managed by the carbon-atom counts because a few divisions (around C_8 to C_{18}) are heavily overloaded and many divisions are sparsely populated. The ideal distribution is shown in Figure 2; here there are only ten divisions, and all are almost equally populated. The origin of these powerfully discriminating digits is explained in the next figures.

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* U.S. Army Biological Laboratories.

** Wiswesser, W.J. 1956. Literature sources of mammalian toxicity data, with special emphasis on tabulating machinery applications, p. 77. In: A key to pharmaceutical and medicinal chemistry literature. Advances in Chemistry Series 16, American Chemical Society, Washington, D.C.
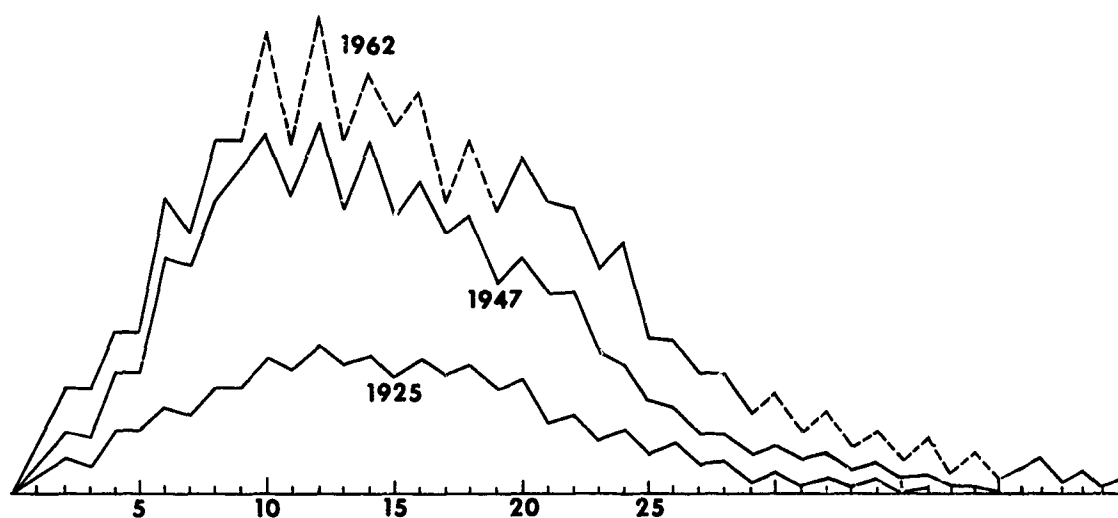
Figure 1.   C-Atom Distribution in Chemical Abstracts Indexes.
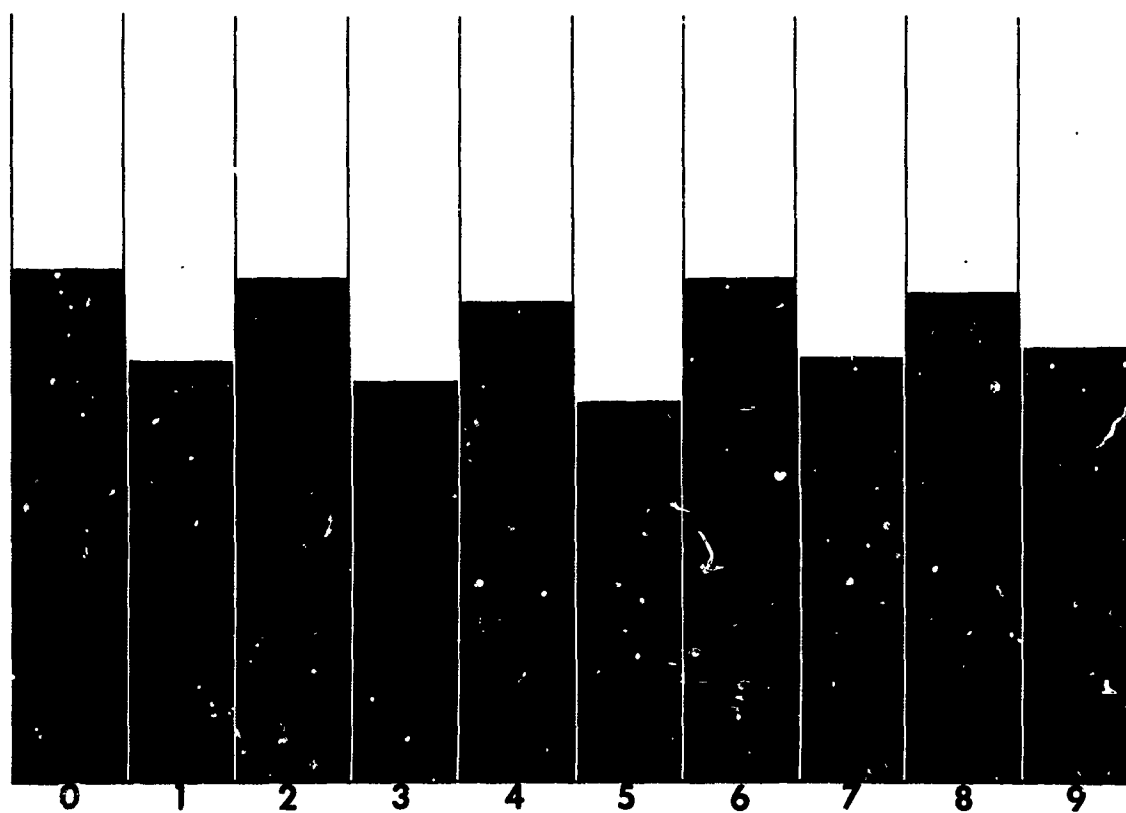

Figure 2.   C-Units Distribution in Chemical Abstracts Indexes.

Figure 3 shows how the various C-atom-counting divisions are merged in a very obvious manner — according to the <u>units</u> digit alone — to yield ten nearly equal "piles" of formula descriptions. The very small 0, 1, 2, and 3 counts are bolstered by the large 10, 11, 12, and 13 counts — and the 20, 21, 22, and 23 counts beyond the heavily populated teens. The figures used to make this diagram were taken from the 1962 Chemical Abstracts index. Figure 4 shows how the markedly different distribution of original C-atom counts in the 1964 Crops file yields a very similar merged pattern.

Figure 5 shows the attractively leveled distribution that is obtained when H-atom counts are merged in a somewhat different way. All indexers know that the odd or even nature of the hydrogen-atom count is dependent on the odd or even valence sum of all other atoms; thus odd-H counts never can be used to index neutral molecules containing zero or an even number of halogen, N, P, B, and related atoms. Likewise even-H divisions are not used when 1, 3, 5, 7, or 9 such odd-valent atoms are present. This poor sorting efficiency is corrected by adding the <u>tens</u>-count to the <u>units</u>-count of H-atoms (Figure 5) with data from the Crops file.

Figure 6 shows the much poorer distribution of this same 1964 Crops file when sorted by the total number of remaining atoms — all except C and H. However, this natural T-atom count still has much usefulness because the dominant range is well within the allowed ten divisions. Just one modification appeared during the ten years of experience with this T-measure; the "zero" division for primitively simple C-H or C-H-O compounds now is combined with the "ten-and-more" division for all other compounds.

Figure 7 shows how a ten-year sampling of random Chemical Abstracts Formula Index selections was divided by the original "atomic class" or A-digits. The well-populated 0, 1, 2, and 3 divisions contain just those compounds that have 0, 1, 2, and 3 or more <u>nitrogen</u> atoms with C (H) (O). Simple <u>sulfur</u> compounds fall in the fourth division, next to <u>sulfur-nitrogen</u> compounds in the dominating fifth division. Similarly, simple <u>halogen</u>-(S) compounds fall in the sixth division, next to <u>halogen</u>-(<u>S</u>)-<u>nitrogen</u> compounds in the seventh division. Originally, all other organometallic compounds fell in the eighth "atomic class" division, and inorganics occupied the ninth.

Figure 8 shows the corresponding distribution of the 1964 Crops compounds in the first eight A-digit divisions. Our 0-division is significantly smaller, and our seventh division (for halogen-nitrogen compounds) is larger. Phosphorus compounds have become so prominent during the past ten years that the newly defined eighth A-division is reserved for these alone. If any other (rarer) atom also is present, the compound falls in the last "atomic class" because this total collection remains significantly smaller than the preceding (A8) collection of simple phosphorus compounds. In the more comprehensive <u>CA</u> index, these last two A-divisions are nearly equal.

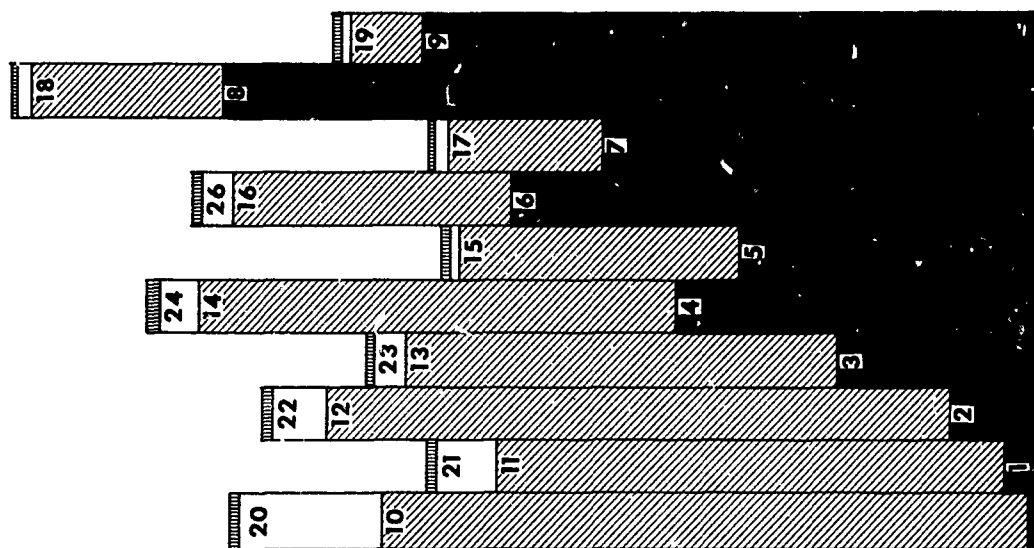Figure 4. Merged C-Atom Distribution in 1964 Crops File.
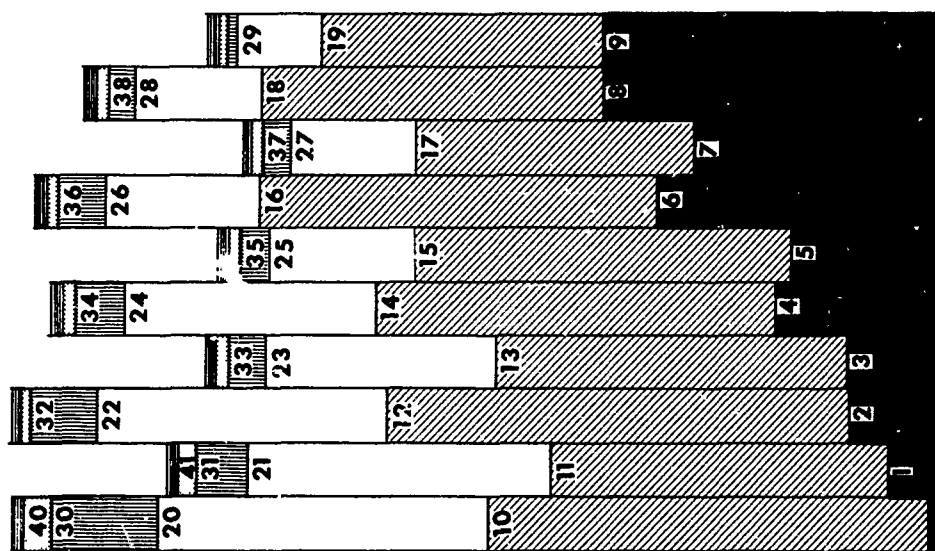

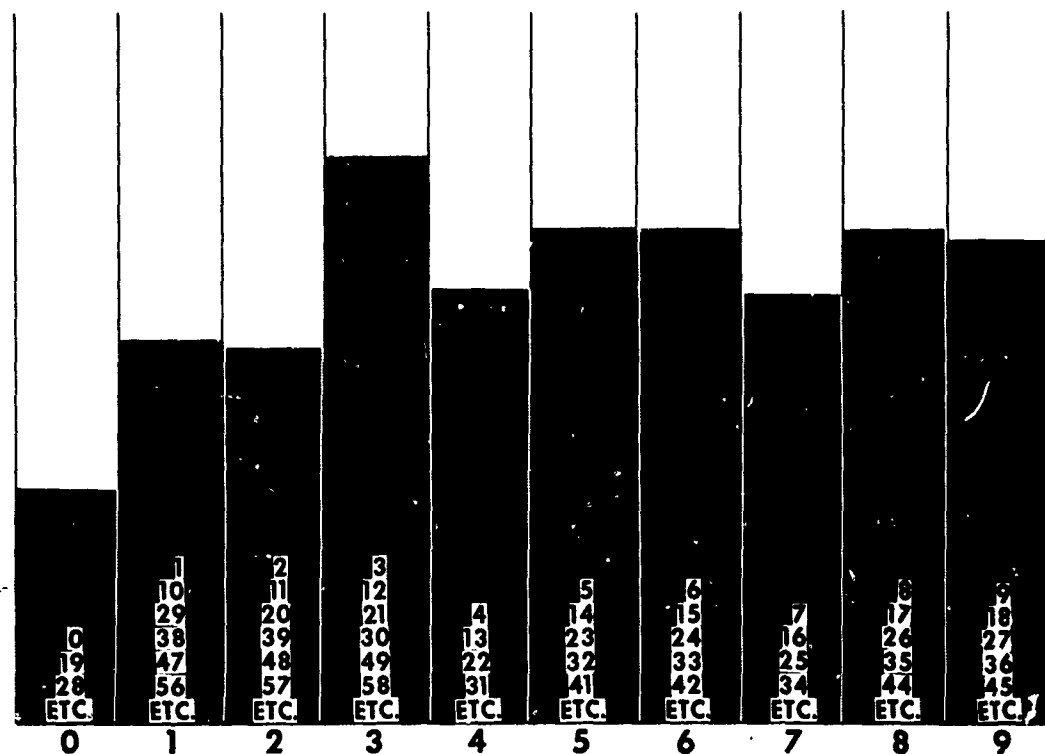Figure 3. Merged C-Atom Distribution in 1962 Chemical Abstracts Index.

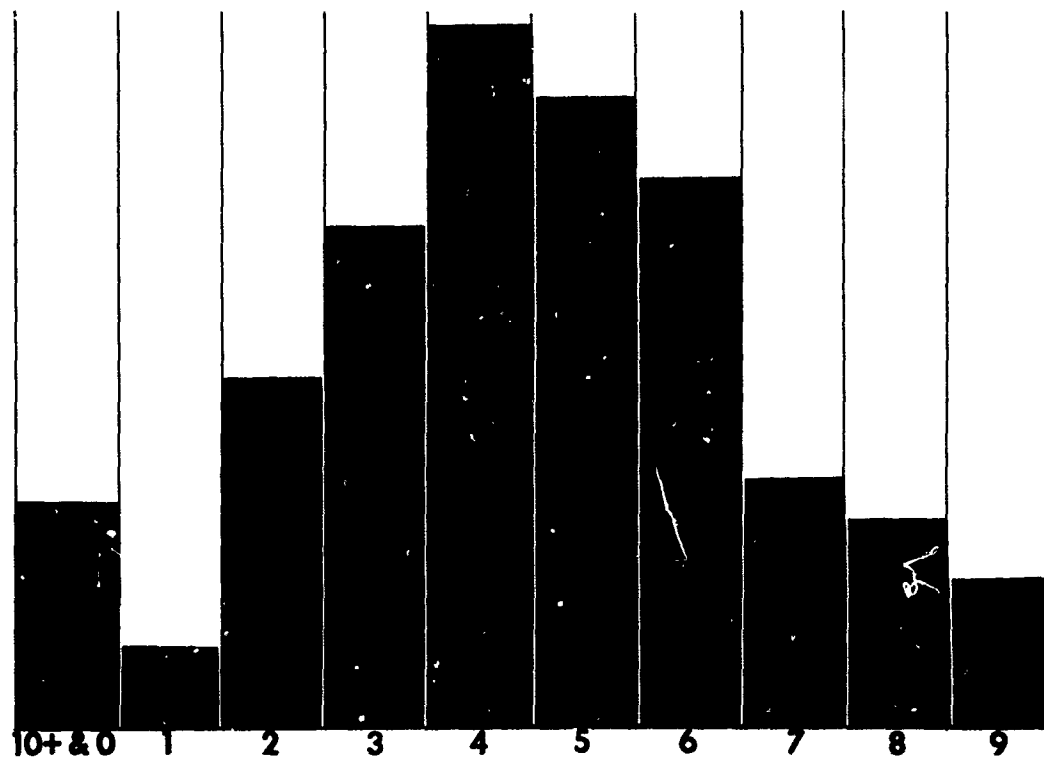Figure 5. Summed H-Digit Distribution in 1964 Crops File.

Figure 6. Total Heteratom (T-Digit) Distribution in 1964 Crops File.
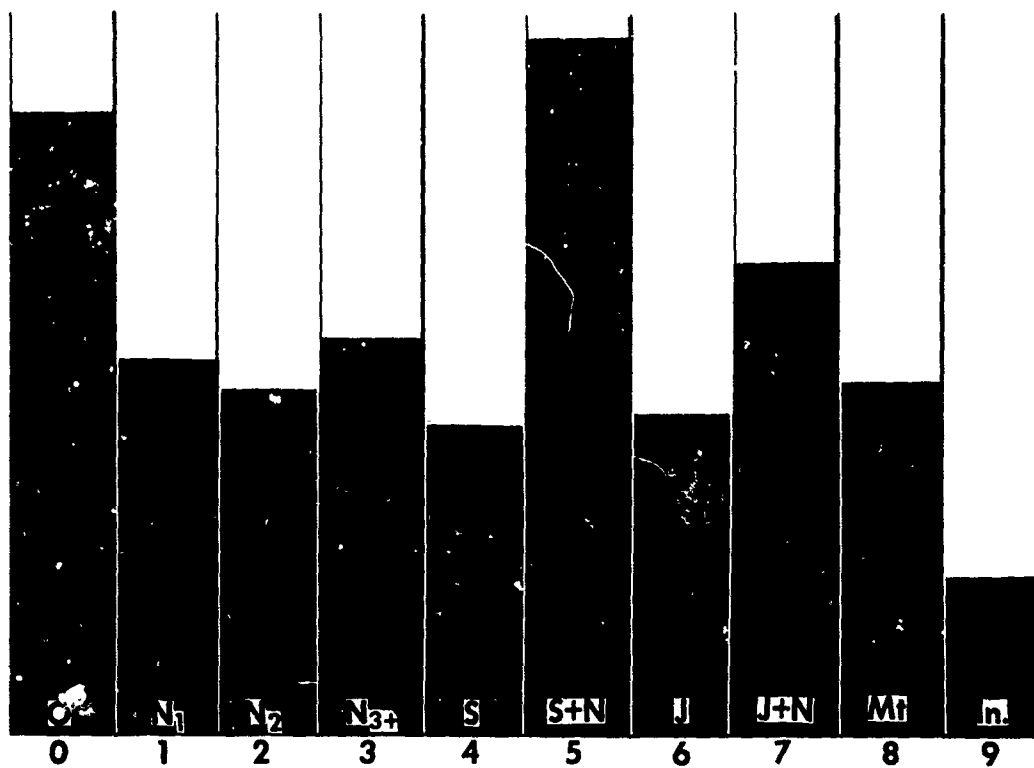
Figure 7. Atomic Class (A-Digit) Distribution in Chemical Abstracts Indexes.
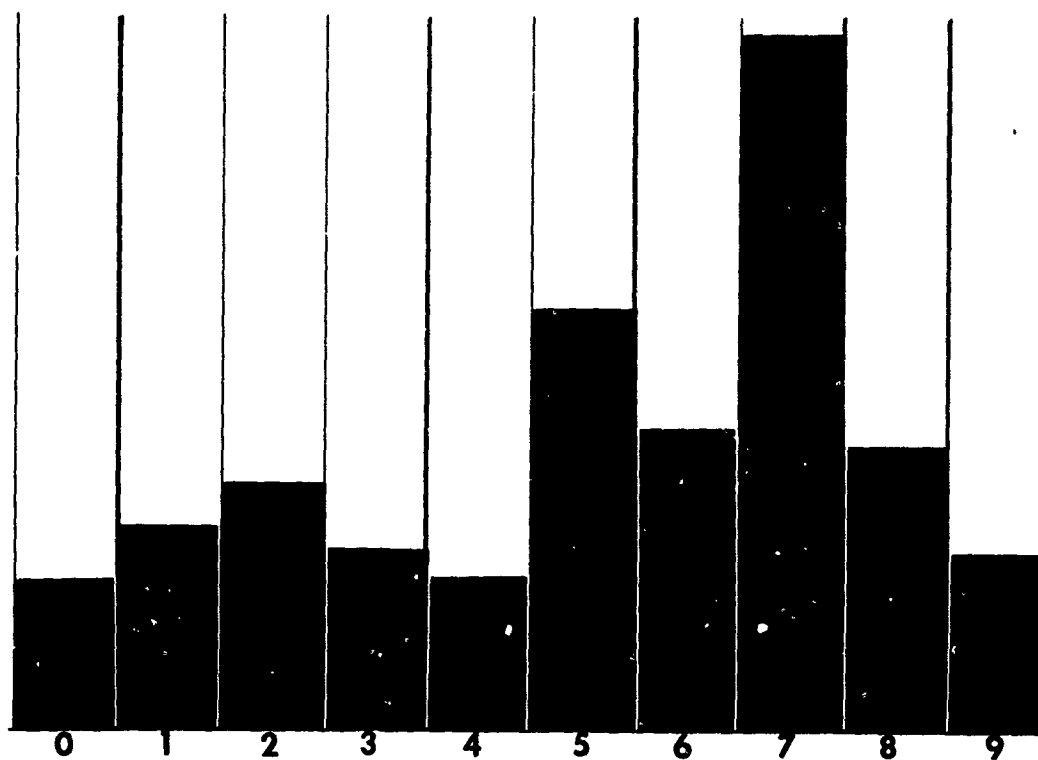
Figure 8. Atomic Class Distribution in 1964 Crops File.

Figure 9 shows how the Chemical Biological Coordination Center (CBCC) file, the nation's largest past collection of biologically tested compounds, is distributed according to fundamental ring structure, as defined by the B-digits. The B-values 0, 1, and 2 mean zero, one, and two or more benzene rings only. (These occur more frequently than all other rings combined, hence justify this special distinction.) The next two B-values bring together structures having a singular or plural count of monocyclic rings of all other kinds; the "plural" count in this and the next set also includes the simple addition of a benzene ring. The next two B-values (5 and 6) similarly bring together structures having a singular (5) or plural (6, with benzene) count of bicyclic rings. The tricyclic and higher structures are much rarer, so that single divisions are more than adequate for their separation from the preceding multitudes.

Figure 10 shows how our 1964 Crops file is classified by these same ring-class definitions; the eighth division — for tetracyclic compounds — is so small that it could be merged with the almost insignificant ninth-ring division — for pentacyclic and higher ring multiplicities. However, the steroid chemists welcome this sharper A8 definition of their important class of compounds.

Figure 11 illustrates the surprisingly efficient sorting power of the A-T-C-H digits alone (without the above B-digit). Successive pairs of columns in this diagram represent the number of compounds found in the largest formula indexes of Lange (L) and Hodgman (H) handbooks. These most prominent divisions are not at all significantly increased by the merging of much simpler A-T-C-H numbers, and the many scattered smaller divisions are constructively associated by the same simpler measures.

B. "DOT-PLOT" DIAGRAMS AND TABLES

Early this year we learned how to take advantage of the UNIVAC computer's special talent to read all possible combinations of its punched-card patterns. There are 64 such binary combinations of six punching positions, and of course only 26 of these have alphabetic meaning. Our "Dot-Plot" program tells the computer to look at the physical pattern of holes in the "picture-frame" part of the dot-plot cards and to use these graphical coordinates to "program" the printing of letters or numbers or marks that are registered in another part of the same dot-plot card. A single card has enough carrying capacity for 40 such marks, to be printed anywhere within the top half of the total picture field (six rows and 30 columns). A second card can extend this capacity to 80 marks and the picture field to another six rows below the first six.
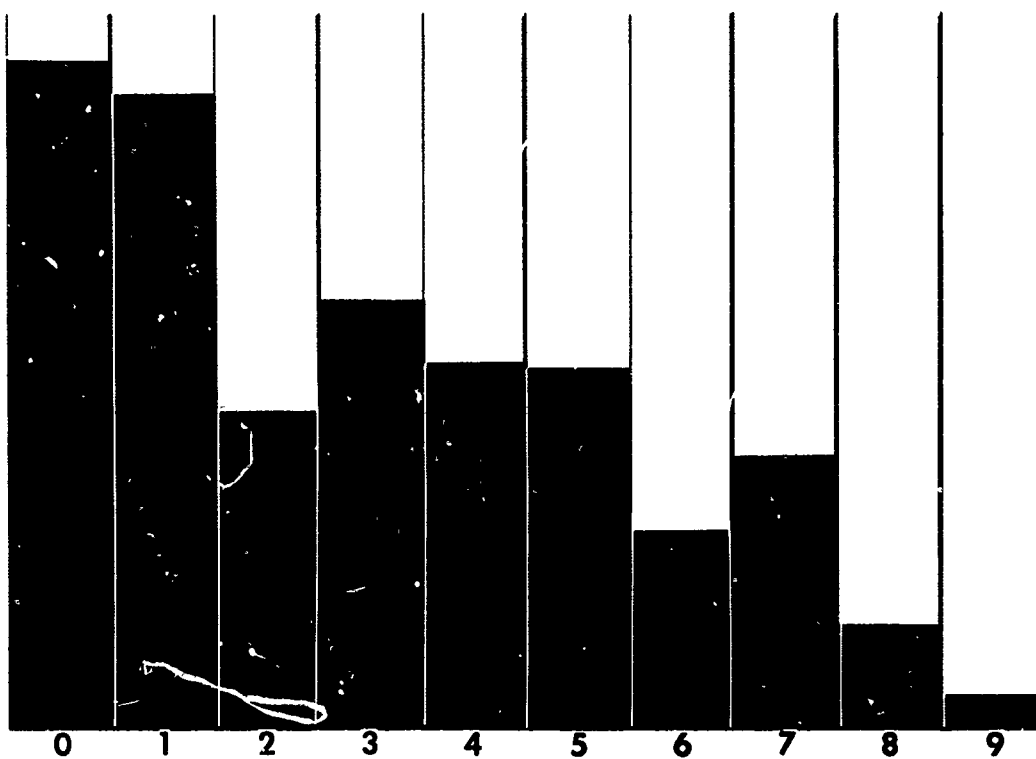
Figure 9. Ring-Class (B-Digit) Distribution in CBCC Catalog.
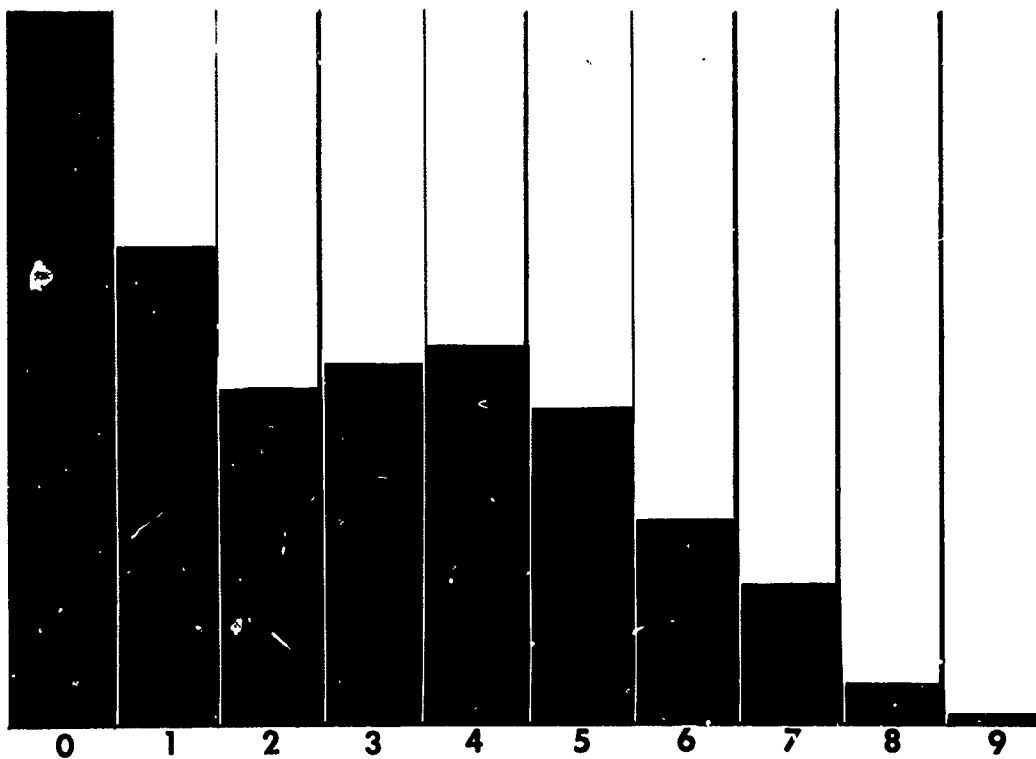
Figure 10. Ring-Class Distribution in 1964 Crops File.

Hundreds of thousands of chemical structure diagrams can be registered on single dot-plot cards when the structure-forming atomic groups are plotted with single printing marks. Examples in our demonstration list include tetracyclic and pentacyclic complexities like cholesterol, morphine, penicillin, and yohimbine. The familiar atomic symbols B, C, F, H, I, N, O, P, and S appear in these diagrams with a few other high-frequency single-letter symbols such as E for Br, G for Cl, M for NH, Q for OH, Z for NH_2, L for the "aliphatic CH_2-links," and D for the "dehydrogenated diatomic :CH- group." These electrically composed diagrams gush out of the line-printer so fast that you will not believe it unless you see it — so we have a demonstration arranged for this conference.

Figure 12 shows how this high-speed graphical printing capability is used to display screening results on six crops (the six horizontal lines) involving eight visual effects (ABscission, CHlorosis, COntact injury, KIll, NEcrosis, STunting, OTher, and summarizing ACtivity — coded in the eight tabular headings) and two dosage rates (the pairs of digits). For example, if the registered chemical's action is slight, moderate, or strong in any of the 96 tabulating positions, the corresponding green-house code numbers 2 (slight), 3 (moderate), or 4 (strong) are printed in the fixed position. Relatively few chemicals show activity in more than ten to 20 tabulating positions, so a single dot-plot card suffices for the vast majority of tested chemicals, and none have overloaded the limiting capacity of 80 plotting-and-printing positions.

Pairs of digits give a logical tabular summary of the activity data of the low dosage (0.1 pound per acre) and high dosage (1.0 pound per acre) when the "low" is oriented to the left of the "high." The same activity-rating numbers are used in both columns, but an activity at the low (left) dosage rate means ten times as much as when entered at the high (right) listing position. Thus the paired activity registration "23" means "2" or "slight" activity at the low rate and "3" or "moderate" activity at the high rate. This over-all organization of screening activity data provides almost instant comprehension of each chemical's "activity spectrum," and we look forward to much easier information retrieval when the 100,000 cards in Crops "File 19 on Visual Effects" are consolidated onto some 10,000 dot-plot cards.
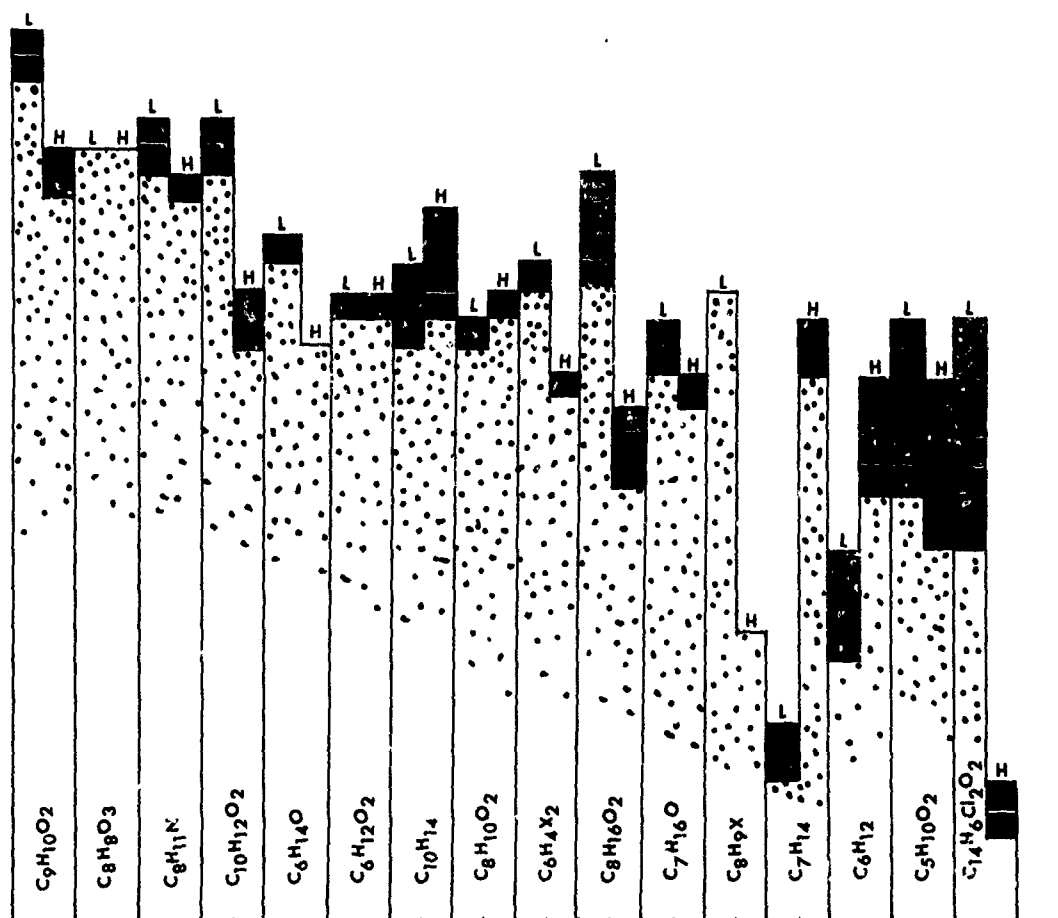
Figure 11. Increase in Largest Formula Divisions Caused by A-T-C-H Merging.

	AB	CH	CO	KI	NE	ST	OT	AC
B	11	12	13	14	15	16	17	19
S	21	22	23	24	25	26	27	29
M	31	32	33	34	35	36	37	39
R	41	42	43	44	45	46	47	49
O	51	52	53	54	55	56	57	59
R	61	62	63	64	65	66	67	69

Figure 12. High-Speed Tabulation of Visual Effects.

XI. THE SYNTHESIS OF NEW DEFOLIANTS

J.K. Leasure*

Tordon (4-amino-3,5,6-trichloropicolinic acid) is a new herbicide
that is extremely active against many species of woody plants, both
broadleaf type and conifers. The outstanding activity of this hetero-
cyclic compound encouraged the synthesis of certain other heterocyclic-
nucleus compounds in a more or less systematic manner.

Since our in-house research had already investigated the pyridine
nucleus rather exhaustively, we decided to employ this knowledge in
the investigation of several other ring systems.

The work follows two simultaneous approaches for each heterocyclic
nucleus. The first is the synthesis of specific structure by a series
of known chemical procedures, which should result in reasonably predict-
able structures of fair purity.

The second approach is the synthesis of compounds by a series of
steps — one or more of which are relatively nonspecific polyhalogenation.
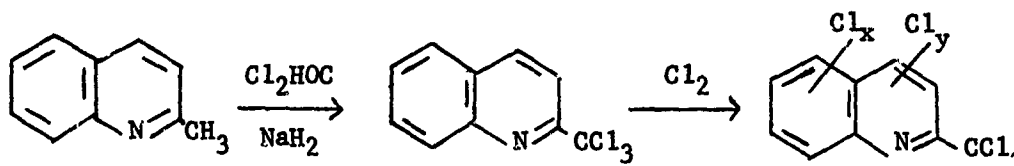
The separation and identification of the components of these mixtures
is tedious and difficult work, but our past experience indicates that it
pays to do the work. Once the course of a chlorination is known and
methods worked out for the separation of pure components, whole groups
of new and interesting derivatives are available for testing.

Using pyrimidine as an example, these techniques could yield these
two compounds:

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* Dow Chemical Company.

94
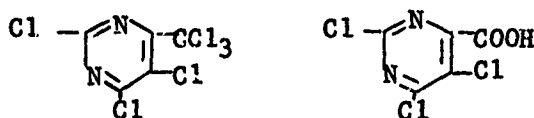
A number of substitutions at the active positions of each of these probably occur to several of you here. As many of these as are practical are being made, and their activity is being compared with that of similar pyridine ring compounds.

Other heterocyclic ring systems under investigation included the following:

quinaldine
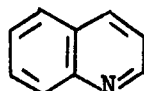
pyrazine

purine
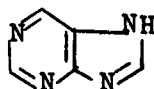
quinoxaline
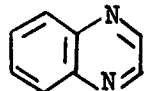
thiazole

indole
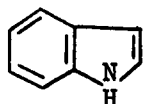
thiophene

Although this contract has been in operation for only a few months, more than 25 compounds have been prepared, submitted to Fort Detrick, and tested at the Dow laboratories. Some of these compounds are active at relatively low rates, and lead us to believe that this program will be productive, and that we are no longer shooting entirely in the dark.

XII. NIAGARA SYNTHESIS AND SCREENING PROGRAM

Joe R. Willard*

Our synthesis and screening program was initiated late in June 1964 when we were informed that our proposal had been accepted. Organization for accomplishing the proposed program was quite simple and virtually without modification of our normal departmental organization. Dr. Sanford Young, under the general supervision of Dr. Paul E. Drummond, our Manager of Organic Synthesis, is responsible for the planning and execution of the synthesis program. The biological screening and evaluation programs are the responsibility of Dr. Kenneth Hill, under the general supervision of Dr. Kenneth Dorschner, our Product Manager for herbicides. Both of the men are attending this conference and I now know, well-known to at least the plant physiologists among you.

A support group, our Chemical Services Group, under my direct super-vision, is responsible for preparation of intermediates and certain of the final products, and for the physical handling of samples for shipment to Fort Detrick. When the time arrives for the larger evaluation samples, this group will also be responsible for their preparation.

Our basic screening procedure for defoliation using the Black Valentine bean is essentially the same as several of you have already described at this and the previous conference, thus I will not take your time to elabor-ate on it. We also will simultaneously submit compounds to our company screening program for potential use as agricultural herbicides. Details of our evaluation have not been completely worked out. However, these details will be based upon studies using (i) two deciduous species — silver maple and English boxwood; and (ii) two broad-leaved evergreens — euonymus and California privet. The size of our program is presently relatively small so that shipments will probably not be made more frequently than biweekly.

Our original proposal suggested six areas that we feel to be of interest as potential defoliants on the basis of our assessment of the biological properties of the compounds.

Compounds from one of these classes, the dichloromaleimides, were under development several years ago as agricultural herbicides and as defoliants, but development was discontinued when it was found that the economics of the use were not favorable. Our interest in this group was rekindled in recent months by the discovery of an additive that very materially increased the potency of the compounds. We are not yet in position to say positively that this effect will reactivate our development program.

* Niagara Chemical Division, FMC Corporation.

The other five classes proposed are so new to our programs that I am
not at liberty to discuss them in any detail today. Generally, these com-
pounds lack the selectivity desirable in a commercial agricultural herbi-
cide, but do have the very desirable traits of low-order toxicity to
mammals (most have acute oral toxicities in excess of 10 grams/kg) and
short residual life in the soil (degraded overwinter). Due to the
relatively limited selectivity of the classes, one compound has been
field tested this season as a brush killer, with, I am very pleased to
report, most encouraging results.

XIII. THE HOOKER SYNTHESIS AND SCREENING PROGRAM

Edward D. Weil*

The Hooker Chemical Corporation is a newcomer to the Fort Detrick defoliant program. Our contract research began on the first of June of this year, so most of what I have to say today will be by way of introduction.

Our interest in the defoliation and herbicide field goes back many years. Hooker, in 1956, acquired by merger the Oldbury Chemical Company, a U.S. pioneer in the agricultural use of sodium chlorate. Since the merger, Hooker has expanded the sodium chlorate manufacturing facilities, notably by expanding our plant at Columbus, Mississippi. A significant amount of our chlorate is sold to formulators for the preparation of cotton defoliants, as well as for soil sterilant use.

Chemical and biological research under the Fort Detrick contract is being conducted at our corporate research laboratory at Grand Island, New York, just across the river from our Niagara Falls plant. At this location, in addition to our chemical laboratories, we have a recently-built research greenhouse and 50 acres of good farmland suitable for small-scale field plot experiments.

The personnel assigned to our Fort Detrick contract program are as follows: Dr. Edward D. Weil as project co-ordinator; Dr. Dale W. Young, plant physiologist in charge of the biological testing; Dr. Jim Hodan, supervisor of our Organophosphorus Group, who is in charge of the synthesis; Dr. Richard D. Carlson, organic chemist performing the synthesis work; Mr. Gerald Hoover, a trained and experienced synthesis technician; and Mr. Ronald Allen, a Bachelor of Science from Cornell Agricultural College, who carries out the screening. Also contributing materials to the program will be our Agricultural Chemicals and our Organophosphorus Research Groups.

Our research proposal which led to the present contract program has a rather well defined scope and is backed up by a certain amount of data already on hand from our herbicide screening program. We have proposed the systematic investigation of the area of aliphatic phosphine oxides and closely related compounds.

A word of explanation regarding the nomenclature is in order. We do not mean to encompass within the scope of our principal interest such compounds as the phosphoramides, phosphonamides, and the like, which are sometimes unsystematically named as phosphine oxides. We are primarily interested in the true phosphine oxides, that is, those having the central phosphorus atom bonded directly to carbon.

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* The Hooker Chemical Corporation.

It would be premature to go into any structure/activity discussion based on our work prior to, or during the brief course of our contract. I should hope that we will have the opportunity of doing so at a similar conference next year. Suffice it to say at present that a substantial degree of very rapid foliar activity, conspicuously desiccant in nature, resides in simple trialkylphosphine oxides. These simple compounds are quantitatively much more active, but qualitatively similar in regard to visible effects on the plants to the known trialkyl phosphates and the dialkyl alkanephosphonates with which the Fort Detrick group has experimented.

Although trialkyl phosphates and dialkyl alkanephosphonates might be able to act as alkylating or phosphorylating agents toward reactive groups in plant tissue, no such modes of reactivity can reasonably be ascribed to a simple trialkylphosphine oxide. A well-known characteristic of simple trialkylphosphine oxides is that of remarkable chemical stability to most acids, bases, oxidants, and reductants. However, because of the high degree of polar character in the phosphorus to oxygen linkage, the oxygen atom is electron rich and readily shares an electron pair with protonic acids, metal cations, and a variety of Lewis acids. It has also recently been shown in Russian work that in the presence of a suitable single electron donor such as potassium metal, the phosphine oxide structure can sustain an unpaired electron, detectable by EPR, on the phosphorus atom. Furthermore, the trialkylphosphine oxides have their hydrogens rendered somewhat acidic by the strong over-all electronegative effect of the P-O group. With these various chemical modes of behavior possible, we will find it interesting to compare the experimental data from a systematic exploration of this area against the various hypotheses concerning mode of action. We would like eventually to be able to carry out a mathematical analysis of the biological data versus the partition coefficients and "sigma-plus" values for the substituents in the manner recently published by Hansch et al.* The predictive value of such a quantitative correlation would, hopefully, reduce the element of chance in the selection of candidate compounds.

Our synthesis methods do not require extensive description. Many phosphine oxides or their precursor phosphines can be prepared on a laboratory scale by attaching the chosen organic radicals to phosphorus by use of organolithium or Grignard reagents, starting with the phosphorus in the form of phosphorus trichloride, phosphoramidochlorides, phosphorus oxychloride, and the like. We can anticipate, however, that more practical and economical large scale methods of manufacture of organophosphine compounds can be worked out for any useful compounds to come out of this research. One of the technical breakthroughs required for such commercial development of the organophosphine series was the development at Hooker of a practical electrolytic phosphine cell. A lengthy series of U.S.

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* Hansch, Corwin; Fujita, Toshio. 1964. ρ, σ, π analysis. A method for the correlation of biological activity and chemical structure. J. Amer. Chem. Soc. 86:1616-1626.

patents was issued last year to Hooker Chemical describing some of the technology involved in the design and operation of a cell for converting phosphorus directly to phosphine. Hooker is now manufacturing on a commercial scale a reaction product of formaldehyde and phosphine, THPC, used for flame retardant treatment of cellulose; we expect to employ some of the chemical knowledge that we have gained in this area of chemistry for the benefit of the Fort Detrick program.

In regard to testing procedures, we are following the recommendations of the Fort Detrick group in screening on Black Valentine beans. In addition to spraying the chemicals in acetone, we have also decided to evaluate an aqueous solution or aqueous emulsion of the same chemical in a parallel test. This doubles the work of spraying and recording results, but we feel that since the ultimate carrier is likely to be water in any case, the performance of the chemical in aqueous formulation is important to determine. We have noted already rather significant deviations between acetone and water applications, higher activity being shown with water in a number of cases.

In addition to our defoliant tests, Dr. Young is running a pre- and post-emergence test on each chemical at 8 and 4 pounds per acre respectively, on a wide selection of crops.

For secondary testing, Dr. Young has planted a suitable number of spruce, elm, euonymus, and pyracantha on our experimental farm.

We are optimistic about our program, especially because among the materials we have already tested, we have seen an encouraging degree of rapid nonselective foliar destruction.

In conclusion, I would like to thank Dr. Minarik and his group for their indispensable assistance in helping us launch our research program.

XIV. THE DEFOLIANT CONTRACT PROGRAM AT AMERICAN CYANAMID

Richard J. Magee*

Cyanamid's contract is for the synthesis and evaluation of compounds possessing potential as defoliants, herbicides, and/or foliar desiccants. The contract program is being carried out at the Cyanamid Agricultural Center by chemists and biologists who are regular members of our herbicide research staff.

I will discuss in a general way the basis from which our contract proposal was formed, and then specifically one of the chemical areas in which we are working. Since this is our first participation in this conference, I will take a few minutes to tell you about our Agricultural Center and how our herbicide leads are discovered.

The Agricultural Center is located on a 640-acre site in central New Jersey, four miles from Princeton. Some 500 persons are employed at the Center, about half of whom are engaged in research and development. Research is carried out not only in the areas of crop protection and crop growth but also animal health and nutrition. A full range of activities is carried out including evaluation, use of experimental chemicals under farm conditions, chemical synthesis, process development, formulation, analytical research, metabolic studies, and mammalian toxicology. Included among special equipment are climate-control chambers and plant-culture rooms.

An important element in Cyanamid's agricultural research program is a central file of chemicals known as the CL File. This sample file provides the compounds for evaluation in the biological research programs. By means of this file all chemicals synthesized or acquired for any purpose at any Cyanamid research location are made available for testing. At present there are more than 60,000 compounds in this file.

Several thousand compounds a year are tested as herbicides. Compounds active in the herbicide screening test proceed to secondary evaluations in post- and pre-emergence applications where their effectiveness and other properties such as systemic action, selectivity, and plant-growth effects can be further investigated.

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* Dr. Magee is a group leader in the Chemical Research and Development Laboratories, Agricultural Division, American Cyanamid Company, where he has been concerned with pesticide synthesis and development since 1957. He is project supervisor of the Cyanamid contract defoliant program, DA-18-064-AMC-244A.

The leads chosen for our contract program had been evaluated in such tests. Three different chemical types were selected. All were considered of possible commercial interest, but more importantly, each was selected because of its defoliant properties (Table 1).

TABLE 1. LEADS SELECTED FOR CONTRACT PROGRAM

Compound	Pre-emergence Activity (≤5 lb/A)			Postemergence Activity (≤5 lb/A)				
	Monocot	Dicot	Soil Residual Activity	Monocot	Dicot	Systemic	Desiccant	Defoliant
A	+	+	-	+	+	+	+	+
B	+	+	?	+	+	+	-	+
C	+	+	+	+	+	-	+	-

All showed effectiveness in both pre- and postemergence application against both broad-leaf plants and grasses. Two were known to behave systemically; two were very effective desiccants; and two had appreciable defoliant activity.

Today we will discuss the third area, a series of s-tetrazines, which possesses unique chemical and biological properties.

s-Tetrazines have been known for seventy-five years. Many references are to 3,6-diaryl tetrazines. Representative syntheses[1,*] are shown in Figure 1.

The ready oxidation of the dihydrotetrazine, often an intermediate in tetrazine syntheses, is easily observed by the formation of the bright red tetrazine color.

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* See Literature Cited, page 107.
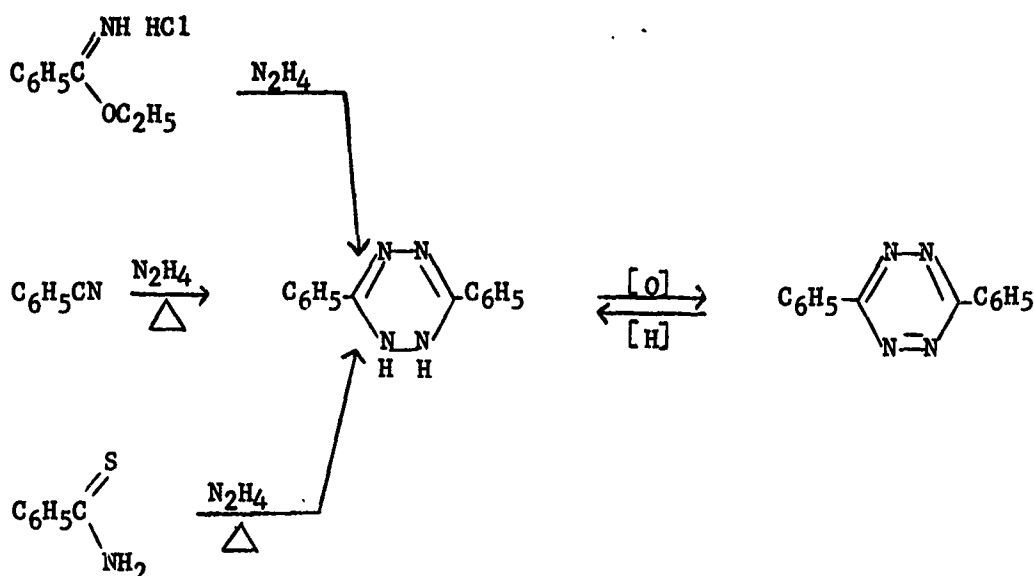
Figure 1.   Representative Synthesis of s-Tetrazines.

Special methods have been developed for selected types of 1,2,4,5-tetrazines. Monoaryl tetrazines, e.g., may be synthesized by the method of Grakauskas, et al. [a] shown in Figure 2.
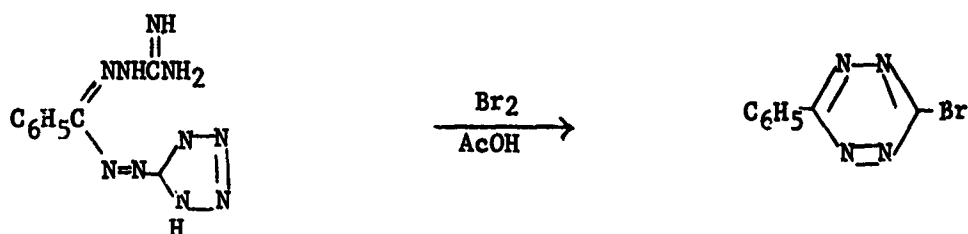
Figure 2.   Representative Synthesis of Monoaryl Tetrazines.

3,6-s-Tetrazine dicarboxylates may be prepared by the base-catalyzed self-condensation of ethyl diazoacetate, followed by oxidation.[3,4]

s-Tetrazine itself can be prepared by decarboxylation of 3,6-s-tetrazine dicarboxylic acid, as shown by Hantzsch and Lehmann[5] in 1900 and by Spencer et al.[6] Huisgen[7] proposed a 1,3-dipolar ion mechanism for this reaction.

Cases of special chemical interest are found in those compounds that may be formally considered to be dihydrotetrazines substituted 3,5 with hydroxy or mercapto groups. These compounds, considered in their keto forms, are referred to in the literature as "p-urazine" and "dithio-p-urazine" as shown in Figure 3.
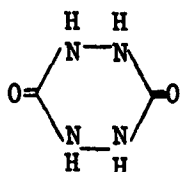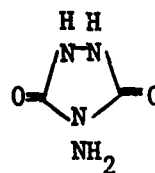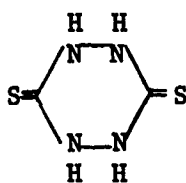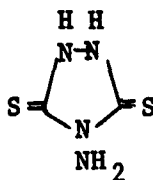
Figure 3. Structural Formulas for "p-urazine" and "dithio-p-urazine."

Work by Dr. Albert W. Lutz, in our laboratory, and others indicates that in most if not all instances the compound characterized as "p-urazine" (I) is either biurea (II) or 4-aminourazole (III). Furthermore by at least some routes the product thought to be "dithio-p-urazine" (IV) is in reality its isomer 4-aminodithiourazole (V).[8] A synthesis of authentic dithio-p-urazine has been published.[9]

This program has yielded some interesting compounds with respect to herbicidal action. The extremely high contact activity of compounds such as FD 82,012, shown below, is of interest.
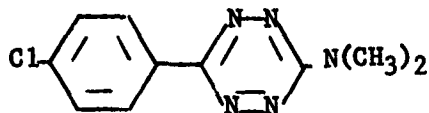
FD 82,012

This compound is highly active against both monocots and dicots and has a desiccant action. Furthermore, it is extremely rapid in its effect. Certain species are susceptible to applications of a few ounces per acre (Table 2).

TABLE 2. FOLIAR HERBICIDAL ACTIVITIES
(Greenhouse tests; data taken four weeks after application)

Herbicidal Index[a]

p-Cl-C$_6$H$_5$ —〈 N—N 〉— N(CH$_3$)$_2$ [b]   Diquat[c] · 2 Br$^-$

Test Plant	Rate of Application (lb/A)					
	2.5	0.5	0.1	2.5	0.5	0.1
Canada thistle	9r	9r	t	9	9r	9
Chickweed, mouse-ear	9	9	7g	8g	5g	g
Mustard	9	9	0	9	9	0
Pigweed	9	8	2s	9	9	8
Purslane	9	9	0	9	0	0
Barnyard grass	9	2	0	8	0	0
Crabgrass	2	0	0	9	tg	0
Wild oats	8	mg	0	0	0	0

a. Herbicidal Index.   s = severe injury
    9 = 100% reduction in stand   m = moderate injury
    8 = 85-100% reduction in stand t = trace-slight injury
    7 = 70-85% reduction in stand   g = growth retarded
    5 = 50-60% reduction in stand   r = regrowth
    2 = 20-30% reduction in stand
b. Applied in 75% acetone; no surfactant.
c. Applied in water; no surfactant.

For the tetrazines investigated prior to the contract program, the observed activities permit no ready correlation with structure. There is one possible exception to this statement. The more active compounds appear to be those most difficult to prepare.

In summary, our contract program aims to exploit several attractive leads selected from our regular herbicide program. One such compound is a substituted s-tetrazine. Under the contract we hope to demonstrate the usefulness of such compounds as defoliants and to synthesize analogues, hopefully that are readily manufactured and highly effective.

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XV. CROPS DIVISION SCREENING PROGRAM

Robert A. Darrow*

The present U.S. Army Biological Laboratories defoliation program was implemented in July 1962 with the establishment of a synthesis and screening contract with Pennsalt Chemicals Corporation and an in-house screening program for candidate defoliants. At that time small contracts were in effect with the Illinois State Geological Survey, under the leadership of Dr. Glenn Finger, for the preparation of fluorinated aromatic compounds and with Ansul Chemical Company of Wisconsin for the preparation of analogs of cacodylic acid.

Two additional synthesis and screening contracts were established by 1 January 1963 with Ethyl Corporation and Monsanto Research Corporation.

The vicissitudes of federal support for the contract program have led to some reductions in the size of these initial three contracts. Since July 1963, the earlier contracts with Illinois State Geological Survey and Ansul Chemical Company have been discontinued. During the past year there has been a trend toward reduction in funding of the large contracts and the addition of small contracts for synthesis in specific chemical areas by knowledgeable research teams. Thus the synthesis effort has achieved a broad base of search patterns in many groups of compounds in the attempt to find candidate defoliants, desiccants, and herbicides. We now have a total of nine synthesis contracts with all but one contract incorporating screening tests by the contractor.

I wish to report briefly on the two contracts that have terminated since our last Defoliation Conference. Four classes of fluorinated compounds were examined in the Illinois State Geological Survey contract: phenoxyacetic, benzoic, phenyl carbamate, and N phenyl glycine hydrazides. Specific attention was given to methoxy-substituted benzoic and phenoxyacetic acids. Earlier work at Fort Detrick had pointed to the efficacy of 2-chloro-4-fluorophenoxyacetic acid as an herbicide and had stimulated further interest in the fluorinated compounds.

Under the Ansul contract some 81 preparations of arsenic compounds were submitted to U.S. Army Biological Laboratories. Of these, 46 were arsinic acids; 32 were arsonic acids; and three were intermediate arsenoso derivatives. The contract was expanded beyond the synthesis phases to include a subcontract with the Wisconsin Alumni Research Foundation in a three-phase study of the fate of cacodylic acid in soil and plants. A high proportion of the arsinic acid preparations submitted has shown biological activity comparable to that of cacodylic acid.

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* U.S. Army Biological Laboratories.

Turning more directly to the screening program of Crops Division, it has been stated that with the initiation of the present contract search for new and better defoliants a concurrent defoliation screening program was initiated in-house. J. Ray Frank, who at that time was in the military service, was placed in charge of the screening phase. Coordinate in-house screening tests were conducted on 14-day-old Black Valentine bean plants to test defoliant, desiccant, or herbicidal activity of compounds shown to be active in the contractor screening program or in the 6-plant primary screening program conducted by Dr. DeRose at Fort Detrick. During the early phases of this program, compounds with a rating of ten or higher in the DeRose primary screen were evaluated for defoliation activity. Later, a threshold value of 15 on the scale of 6 to 24 was used in selecting compounds for defoliant screening.

In turn, compounds showing a moderate or higher level of activity as defoliants, desiccants, or herbicides in the 14-day bean test are subjected to secondary screening on woody plant seedlings in greenhouse tests to be described by Ray Frank. Active compounds in this screening program on woody plant seedlings are carried into a field testing program to be described by Kenneth Demaree.

A general resume of the defoliant and herbicidal activity of our Fort Detrick catalog of chemicals has been furnished under a screening contract with Bio-Search and Development Company of Kansas City, Missouri. Chemicals were selected on the basis of defoliant or herbicidal activity on herbaceous plants and rescreened under this contract on seven woody plant species. The following quotations from the final report of this contract give a synopsis of the defoliant ratings of the chemicals available up to the time of our present contract program:

> Basically only the deciduous species (Chinese elm, black locust, willow, and dogwood) responded to a true defoliant response from active chemicals. Black locust was the most responsive to defoliant activity. Chinese elm was somewhat slightly more difficult to defoliate and frequently responded by desiccation rather than defoliation. Willow, likewise, desiccated somewhat readily and was particularly useful in measuring phytotoxicity. Willow also was useful in that it responded readily to various growth effects, such as epinasty, production of adventitious roots and so forth.

> Generally speaking, the evergreens (spruce, pine, and hemlock) were unresponsive to defoliation. In all probability the evergreens are not morphologically receptive to defoliation due to the probable absence of a pulvinoid area.

> Phosphate-type compounds such as tricresyl phosphate, triethyl phosphate, and so forth were relatively inactive. Of the earlier members studied probably endothal was the most promising of the

materials tested. The activity of this type of chemical (endoxo-
hexahydrophthalic acid) is quite distinct resulting in very high
defoliation on the deciduous species and a mixture of defoliation
and desiccation of the evergreens.

Chlorophenyl dimethyl urea derivatives and dichlorophenyl urea
derivatives gave high total activity, but this was normally more
desiccant than defoliant action.

Certain of the dinitro phenol - type compounds also gave high
total activity with the bulk of the action being desiccant rather
than defoliant.

Benzoic acid derivatives, generally speaking, were not good
defoliants. 2,3,5-Triiodobenzoic acid was one of the few deriva-
tives giving some degree of defoliation, and this was restricted
to the deciduous species and particularly black locust and willow.
The terminal action from the benzoic acid derivatives is generally
desiccation.

Evaluation of the defoliant and desiccant action of some 65
carbamate derivatives showed the group to be basically inactive.
Occasional compounds gave some slight phytotoxicity, but this was
a rare occurrence and normally restricted to a single species of
woody plant. The above statements apply to both the carbamates
and the thiocarbamates.

Some 25 phenoxyacetic acid - type chemicals were tested and all
were reported as poor defoliants. The group structure, 2-chloro-4-
fluorophenoxyacetic acid, as a group showed active desiccant action
on deciduous plants only. The few phenoxy acetamide structures
tested were inactive. Three or four glycine compounds tested were
also inactive.

A series of s-triazine compounds were shown to have varying
specific effects depending upon specific structure. Too often the
action of the s-triazines terminated in desiccation rather than
defoliation.

The arsenic acid derivatives are in a class by themselves.
They are without question the most active group of the chemicals
tested. The terminal activity is always very high, and whether
it terminated in defoliation or desiccation is probably also a
function of the dosage levels used. The more active arsenic
acids would be the following: diethyl arsenic acid, ethyl methyl
arsenic acid, butyl methyl arsenic acid, methyl propyl arsenic acid,
butyl propyl arsenic acid, and dipropyl arsenic acid. Cacodylic
acid itself is quite active; however, some of the other derivatives
appear to be slightly superior.

A series of tin-compounds were demonstrated to be poor defoli-
ants and/or desiccants. The new chemicals diquat and paraquat
demonstrate a fair degree of activity, however, the action is
prominently desiccation.

In general perspective on our defoliation program, I wish again to call
attention — particularly for the newcomers in our contractor group — to
the desired characteristics of an effective defoliant:

a) Broad Spectrum of Activity: The agent should be active on many
kinds of plants and vegetation with emphasis on woody species.

b) Rapid in Action: One of our speakers has indicated that the
physical changes that result in defoliation or leaf abscission may take
place within a three-day period.

c) Suitable for Application with Air or Ground Equipment: Agent
should be preferably in liquids of high concentration.

d) Nontoxic to Man and Animals: Compounds of moderate or high
toxicity may be included in our screening program on the basis that highly
favorable candidates may be modified through formulation or other methods
to minimize hazards of toxicity.

e) Stable in Storage: Light-sensitive compounds and other unstable
chemicals should be examined, since suitable formulations of such compounds
that are found to be active may insure stability.

f) Effective in Low Dosage: Our screening program has shown a
number of synthesized compounds to have high activity as defoliants,
desiccants, and herbicides at 0.1 pound per acre.

g) Inexpensive: Cost should not be a factor in the initial con-
sideration of candidate compounds.

h) Readily Available or Capable of Manufacture: Complexity of
initial synthesis efforts should not eliminate consideration of a candidate
chemical.

i) Noncorrosive: Some of our more highly active commercially
available desiccants require special handling in current application
equipment. Proper formulation may eliminate such hazards of corrosive
action on equipment.

XVI. CROPS DIVISION DEFOLIATION SCREENING

J. Ray Frank*

Since our last defoliation conference, we have expanded our Defoliant
Screening Program to include nine contractors instead of the original four.
Most of our screening program centers around the contracts submitted by
you contractors, but other commercial and noncommercial sources are supply-
ing candidate compounds. A number of compounds have also been supplied
through the Industrial Liaison Program, Edgewood Arsenal, Maryland, and by
individual chemical corporations.

A. PRIMARY SCREENING

Chemicals received from contractors have been tested on seven-day-old
crop plants, namely, the Black Valentine bean, soybean, radish, morning
glory, oats, and rice. The compounds that are active either in primary
tests of the contractors or in our tests are then tested in our Primary
Defoliation Screening Program. In this screening program compounds are
applied to 14-day-old Black Valentine beans at 0.1 and 1.0 pound per acre.

B. SECONDARY DEFOLIATION SCREENING

Compounds from contractors have been tested on eight species of seedling
trees in our Secondary Screening Program. Eastern hemlock, Norway spruce,
Chinese elm, black locust, red maple, pin oak, California privet, and Scotch
pine were sprayed in the greenhouse with compounds at 1, 5, and 10 pounds
per acre. During the two-year period 42 compounds from this group including
five from the ILO Program were active enough as defoliants, desiccants, or
herbicides to warrant small plot testing under field conditions. I believe
that this number is commendable when compared with the 65 compounds suffi-
ciently active for field testing from the group supplied by various other
sources since 1944.

Some of the main chemical groups that have shown interesting defoliation
or herbicidal activity include the following:

Acetic acids — Phenoxy	Organometallics — tin, germanium
Arsenicals	Phenols
Benzoic acids	Phosphorus compounds
Diols	Picolinic acids
Endothals	Triazines
Fluorides	Uracils
Iodine compounds	Ureas

* U.S. Army Biological Laboratories.

Our primary goal is to obtain a rapid-acting, effective defoliant that will induce abscission of leaves on herbaceous and woody material. We believe that the contracts that are in process have provided and will continue to supply new leads for effective defoliants.

XVII. PENNSALT SCREENING PROGRAM

Herbert Q. Smith*

Our presentation consists of a discussion of our screening work including some aspects of the formulation problem. This will be followed by further data on structure-activity relationships.

In our primary screening program we apply the compound in a suitable formulation to two-week-old Black Valentine Bean plants at 0.1-, 1.0-, and 10-lb/A rates and observe the response for three weeks.

Further work during the contract year showed the effect of formulation on plant response. It is desirable at the outset of this discussion to define terms, because we thought there was some confusion in this regard at last year's symposium. We have summarized the terms in Table 1.

TABLE 1. VARIOUS FORMULATIONS AND APPLICATION MIXTURES USED IN SCREENING

	Formulation	Surface-Active Agent	Application Mixture Carrier	Final State
	Aqueous	-	Water	Solution
	Nonaqueous			
1.	Water immiscible	Yes	Water	Emulsion
	(example-xylene)	No	Oil	Solution
			Acetone	
2.	Water miscible	Yes	Acetone	Solution
	(example-acetone)		Water	Dispersion
		No	Oil	Solution
			Acetone	
	Wettable powder	Yes	Water	Dispersion
	Dust	Yes	Air	Dust

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* Pennsalt Chemicals Corporation.

An appreciable amount of work was done on the effects of carriers on plant response, particularly oil va. water or acetone. The oil used was a proprietary hydrocarbon spray oil which has almost no phytotoxic effect in itself. A number of specific examples can be cited where the use of oil as a carrier gave substantial improvement, no effect, or decreased effect when compared with water and/or acetone. A summary of carrier effects by compound type is shown in Table 2.

TABLE 2.   CARRIER EFFECT ON DEFOLIATION RESPONSE

Compound Type	Oil va. Water (or Acetone), no. of compounds	
	Increase	Decrease or No Effect
Phosphorus	19	16
Sulfones	6	4
Phenols	5	6
Ureas	3	1
Miscellaneous	15	23

The conclusion appears to be that oil will sometimes, but not always, improve the activity of a compound, but the effect is not necessarily predictable.

Also carried out during the contract year was another secondary screening series on woody species. These were Chinese elm, Norway maple, Euonymus japonicus, English laurel and a dwarf bamboo (Sasa pigmy). Applications were at a single 5 lb/A rate. Different formulations of the same compound were also run in these woody tests.

We have found it convenient to employ a simple rating system for our bean plant primary screening. This system is shown in Table 3. The symbolism does not contain a speed of action factor; its purpose is to give a rapid comparison of the degree of activity among large groups of compounds. A structure-activity list, which breaks down the submitted compounds into 65 structural categories with additional subgroups, is constantly maintained, employing these symbols for activity. The high-rated compounds (1^+ or higher) are further maintained in a separate activity list, which introduces speed of action by listing the time for

initial and maximum effect. This high-rated compound list is further
broken-down into 13 major structural categories (with additional subgroups)
that allows very rapid evaluation of screening results obtained from
structural modification of active compounds without the necessity of
referring to original data sheets. As new active structural groups are
discovered, the high-rated list may be easily expanded.

TABLE 3.   RATINGS FOR DEFOLIATION (D),
DESICCATION (d), AND KILL (K)

Rating	% at	lb/Acre
3+	50-100	0.1
3	1-49	0.1
2+	70-100	1
2	1-69	1
1+	90-100	10
1	1-89	10

We turn now to the discussion of further structure-activity relation-
ships. Yesterday, we discussed Endothalls, acetylenics, isothiocyanates,
and ureas.

Figure 1 summarizes the work done in comparing the activity of a
series of phosphite-phosphonates, phosphate-phosphonates, and thiophosphate-
phosphonates. Based on 14 series of three compounds, where R, R', and R"
were held constant and X = -, 0, or S in each series, the phosphate-
phosphonate (X = 0), was likely to be the most active defoliant and
desiccant in each series, but the effect was not entirely predictable.
The totals of each column do not add up to 14 since a clear judgment as
to the most active or next most active structural type could not always
be made.

$$(RO)_2\overset{\overset{X}{\|}}{P}OR'\text{-}\overset{\overset{O}{\|}}{P}(OR'')_2$$

$$R = C_4H_9\text{-}, \quad C_2H_5CHClCH_2\text{-}, \quad (C_2H_5O)_2\overset{\overset{O}{\|}}{P}CH\text{-}, \quad (C_4H_9O)_2\overset{\overset{O}{\|}}{P}CH\text{-}$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad \overset{|}{C_2H_5} \qquad\qquad\quad \overset{|}{C_3H_7}$$
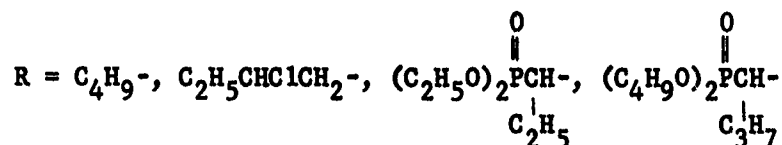
$$X = \text{-}, \; O, \; S$$

$$R' = \overset{}{\underset{\overset{|}{A}}{C}}H\text{-}, \text{ where } A = C_1\text{-}C_{10} \text{ alkyl}$$

$$R'' = C_4H_9\text{-}, \; C_2H_5CHClCH_2\text{-}, \; C_2H_5\text{-}$$

<u>Number of P-Phosphonates Causing*</u>

	Defoliation		Desiccation
	Most Active	Next Active	Most Active
Phosphite	4	5	5
Phosphate	7	1	6
Thiophosphate	2	5	2

* Based on 14 series of three.

Figure 1. Summary of Work Done in Comparing Phosphite-,
Phosphate-, and Thiophosphate-Phosphonates.

A substantial number of covalent iodine compounds defoliated bean plants
at 0.1 lb. Figure 2 lists some of the structural types examined, with an
example of each type. The defoliation observed at 0.1 lb/acre usually
occurs in 14 to 21 days, although it may be faster — in some cases as
early as four days. The leaf assumes a brownish to purple cast and
abscises essentially turgid. There is rarely desiccation at the lowest
rate, but desiccation and kill are common at the 10 lb-rate. So many
of the iodine compounds gave such similar responses that several different
types were tested to determine their reaction rates with cysteine in
aqueous solution over several days, but only slight differences were
discernible.

Example

Ethylenics - A-CI=CI-B $HOCH_2CI=CICH_2OH$

$$\text{Iodoacetics} - \underset{\underset{Y}{|}}{\overset{\overset{X}{|}}{IC}}-\overset{\overset{O}{||}}{C}-Z$$ ICH_2CO_2H

Alkyls - RI $tert\text{-}C_4H_9I$

Higher Oxidation States - RI⟨

Aromatics - ArI

Molecular Complexes - $M \cdot I_2$
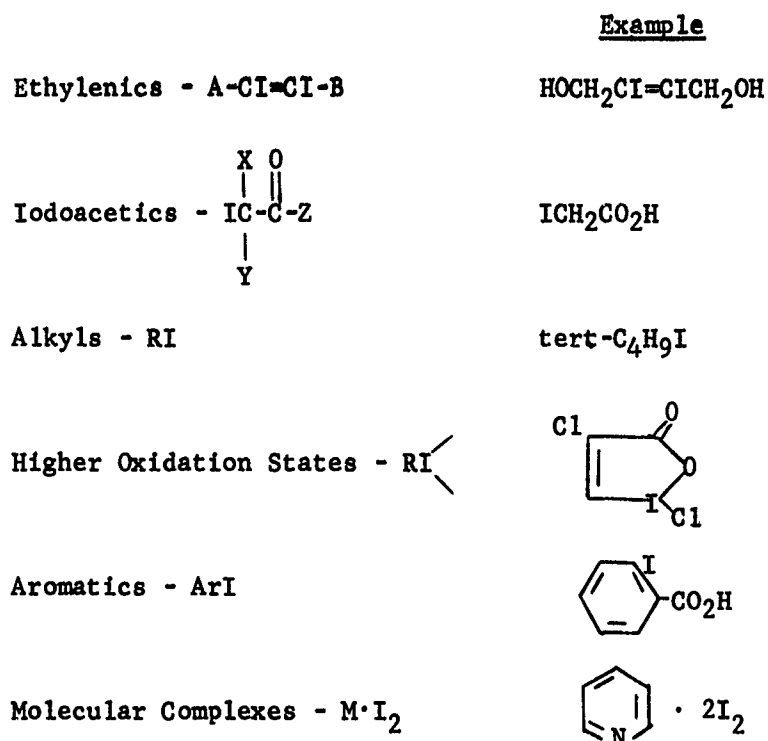
Figure 2. Covalent Iodine Compounds.

The effect of oxidation state on activity is illustrated by the 2-substituted benzothiazoles shown in Figure 3. In the methyl series the sulfoxide is most active, in the butyl series the sulfone is most active, but in the heptyl series, all oxidation states have the same relatively low activity. Conversion of the 2-mercapto compound to its sodium salt gives an active material.

Discussion of structure-activity relationships were also given for the following structural types:

Vinyl sulfones and vinyl phosphonates
Nitrophenols
Halosulfonyl phenols

Benzothiazoles

$$\text{benzothiazole structure: } N=C-S(=X)(=Y)-S-Z \text{ fused with benzene ring}$$
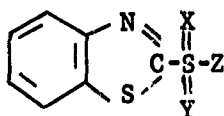
$\underline{X}$	$\underline{Y}$	$\underline{Z}$	Defoliation, lb/A	Desiccation, lb/A
-	-	CH_3	10	10
0	-	CH_3	1	1
0	0	CH_3	10	10
-	-	C_4H_9	10	10
0	-	C_4H_9	10	10
0	0	C_4H_9	1	10
-	-	C_7H_{15}	10	10
0	-	C_7H_{15}	10	10
0	0	C_7H_{15}	10	10
-	-	H	None	None
-	-	Na	0.1	0.1

Figure 3. Substituted Benzothiazoles Causing
Defoliation or Desiccation at the
Rates Indicated.

It appears from our own findings and from what we know of Fort Detrick woody plant tests that, with most or all of the chemical types studied, there are major differences in species susceptibility, the closest correspondence being desiccation response. In the case of defoliation, the conclusion must be either that the mechanism of defoliation varies with the species or that different species have different defense mechanisms for protection against chemical application.

The figures shown in this report do not represent all or even most of the compounds made in the various structural categories. The compounds shown were selected only to illustrate specific structural relationships.

XVIII. ETHYL DEFOLIANT SCREENING PROGRAM

John C. Wollensak*

I will first discuss the results from our Ethyl screening program con-
ducted at Boyce Thompson Institute in general terms, then cover specific
compounds and their levels of plant-response activity.

Of the compounds evaluated in the primary screening at Boyce Thompson
Institute, 26% showed a high level of herbicidal or defoliant activity on
14-day-old Black Valentine bean seedlings. These compounds had a phytoxicity
rating equal to or greater than eight on a scale of eleven, or they caused
abscission of 25% or more of the total leaves. Since the secondary leaves
of the bean plant emerge during the test period, the abscission percentages
must be doubled to give the percentage abscission of primary leaves actually
sprayed with the candidate compound.

Eight per cent of the compounds had a phytotoxicity rating of four to
seven inclusive, or caused defoliation of between 15 and 24.9% of the
total leaves. The remaining compounds showed a lower level of activity.

The several tables of compounds following are materials that showed high
ratings in the primary screen at Fort Detrick conducted under the direction
of Dr. DeRose. Data in the first column of the tables are the screening
results at 0.1 pound of compound per acre; the second column shows results
at one pound per acre. Compounds are rated on a scale of from 6 to 24. In
the secondary screening column, abscission is represented before the slash
and phytotoxicity after the slash. In both the secondary screening columns,
the figures for abscission and phytotoxicity indicate per cent of maximum
effect. For example, tri-n-butyl tin bromide, the first compound, had a
rating in the Army primary screen of 14 of a possible 24 at the level of
one-tenth pound per acre and 21 of 24 at one pound per acre. In the
secondary screen at Fort Detrick, eight tree species, the compound aver-
aged one-quarter of the maximum rating for defoliation of all the species.
The compound received about one-half of the maximum rating, on average, in
phytotoxic effect on all of the species. A similar rating system is used
for the Boyce Thompson secondary screen, which was conducted on four tree
species.

The first table lists tri-n-butyltin compounds having a rating of 16 or
more at the higher rate of application in the Army primary screen. All of
the tri-n-butyltin compounds were highly phytotoxic toward both broad-leaf
and narrow-leaf plants. In general, trialkyltin and triaryltin compounds
are extremely phytotoxic, whereas compounds with one, two, and four carbon-
tin bonds have a low level of activity or are inactive. The activity of a
group of trialkyltin compounds, such as the tri-n-butyltins, is relatively
independent of the fourth group attached to the tin atom.

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* Ethyl Corporation.

The second table shows some of the trialkyltin and triaryltin compounds other than the n-butyl ones. Triethyltin compounds and triamyltin compounds were only slightly less active than the tributyl compounds in the primary screen. Thus, activity of the trialkyltins seems to peak in this region. One tri-n-dodecyltin compound, C_{12}, was tested and found inactive. Triphenyltins, the last two compounds in the table, are very active and appear to have a lower level of mammalian toxicity than the tri-alkyltins tested. Ten of the most effective tin compounds have been prepared on a larger scale for field testing.

The third table lists some mercury compounds which were prepared for the program or were obtained from other sources. Over 70% of the 18 compounds tested had an Army primary rating of 10 or more at one pound per acre. The four compounds shown in the table, all ethylene-mercury salt adducts, were highly effective, having ratings of 16 or more. The discrepancy between the low primary and very high secondary screening results of the last compound is due to the fact that the material was unstable, decomposing into inactive materials. A fresh sample was supplied for the secondary screen. Organomercuries are very phytotoxic, but are not as consistently active as are the trialkyltins. Three of the mercury compounds prepared to date are undergoing field testing.

Table 4 shows the most effective lead compounds. Judging from the organoleads screened, it appears that structure-activity relationships in the lead series are similar to those in the tin series. The trialkyl and triaryl compounds were highly effective, and other lead compounds showed no activity.

Table 5 shows a series of carbamate derivatives of 2-nitro-3,4,6-trichlorophenol. We have made only a limited effort in the carbamate area because of the large amount of literature in the field and because a sizable number of carbamates have already been tested for defoliant application. However, these few materials were prepared because of earlier screening results, and they have shown a consistently high level of activity.

We have screened a large number of phosphorus compounds, many of which were made available from other programs. Some have been effective defoliants, but generally they have been less active than the organometallic compounds tested. Within the organophosphorus class, a few types of compounds have shown consistently high ratings (Table 6). The first two compounds in the table are benzyl phosphonates, and the next three are amides of phosphorus acids having two or three piperidine functions. The other three compounds are an alkyl phosphonate, a phosphoramidate, and a phosphine. These last three classes of phosphorus compounds do not show consistently high results, whereas benzyl phosphonates and phosphorus-piperidine compounds are generally active. A large number of the phosphorus compounds screened had a moderate level of activity, but only a small percentage were as active as the organometallics.

TABLE 1. RESULTS OF TESTING TRI-$\underline{n}$-BUTYLTIN COMPOUNDS
$(C_4H_9)_3SnX$

X	Army Primary Screening,		Secondary Screening (Absc./Phyto.) 5 lb per acre		Field Test
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
-Br	14	21	24/48	44/80	X
-I	11	17	43/57	44/55	-
-OCH$_3$	17	20	-	-	X
-CN	6	20	29/57	-	X
-N$_3$	14	19	-	-	-
-OC$_6$Cl$_5$	16	20	-	25/27	X
-OC$_6$H$_3$Cl$_2$(2,4)	14	20	-	-	X
-OCOCH$_2$Cl	6	19	-	-	-
-NCH=NCH=CH	14	20	-	-	X
-CH$_2$CH$_2$CN	13	20	-	13/59	X
-CH$_2$CH$_2$CO$_2$CH$_3$	7	17	-	-	-
-SSn(C$_3$H$_9$)$_3$	8	20	57/76	-	-
-OSn(C$_4$H$_9$)$_3$	17	22	-	-	X

TABLE 2. RESULTS OF TESTING TRIETHYLTIN, TRI-n-PENTYLTIN
AND TRIPHENYLTIN COMPOUNDS

R_3SnX

R	X	Army Primary Screening 0.1 lb acre	1.0 lb acre	Secondary Screening (Absc./Phyto.) 5 lb per acre Army	B.T.L.	Field Test
C_2H_5	-F	12	18	-	-	-
C_2H_5	-Cl	8	16	-	-	-
C_2H_5	-Br	11	16	-	-	-
C_2H_5	-OH	12	17	48/81	-	-
C_2H_5	-CN	14	20	52/81	-	-
C_2H_5	-OCOCF$_3$	13	17	-	-	-
C_2H_5	-OC$_5$H$_2$Cl$_3$(2,4,6)	15	18	33/76	63/50	-
C_2H_5	-OSn(C$_2$H$_5$)$_3$	21	22	67/81	-	X
C_2H_5	-SSn(C$_2$H$_5$)$_3$	17	19	-	-	-
C_5H_{11}	-Br	11	19	14/52	19/52	-
C_5H_{11}	-CN	10	17	-	-	-
C_6H_5	-Br	15	22	-	-	X
C_6H_5	-NCH=NCH=CH	15	18	-	-	-

TABLE 3. RESULTS OF TESTING ORGANOMERCURY COMPOUNDS

| Compound | Army Primary Screening | | Secondary Screening (Absc./Phyto.) at 5 lb per Acre | | Field Test |
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
$CH_3OCH_2CH_2HgOCCH_3$ (with O double bond)	6	20	-	17/70[a]	X
$CH_3COCH_2CH_2HgOCCH_3$ (with two O double bonds)	7	16	-	31/52[a]	-
$HOCH_2CH_2HgI$	6	21	0/19	19/39	X
$HOCH_2CH_2HgBr \cdot NH_3$	6	9	57/91	-	X

a. At 10 lb/acre.

TABLE 4. RESULTS OF TESTING ORGANOLEAD COMPOUNDS
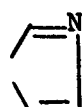
| Compound | Army Primary Screening | | Secondary Screening (Absc./Phyto.) at 5 lb per Acre | | Field Test |
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
$[(C_6H_5)_3PbCH_2CO]_2O$	12	18	29/48	-	-
$(CH_3)_3PbNHCN$	10	16	-	6/9	-
$(C_4H_9)_3PbN$ (pyrrole)	13	21	-	-	X
$(C_6H_5)_3PbN$ (pyrrole)	9	18	-	0/9	-

TABLE 5. RESULTS OF TESTING 2-NITRO-3,4,6-TRICHLOROPHENOL DERIVATIVES
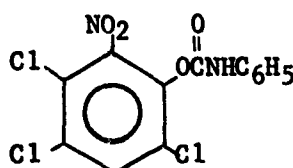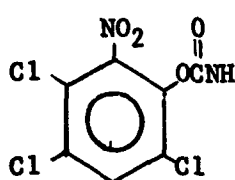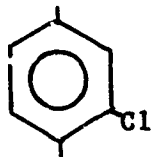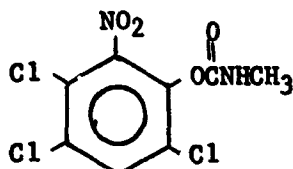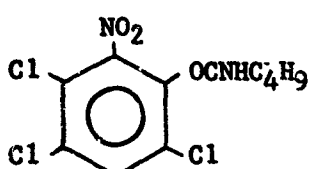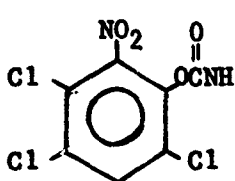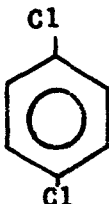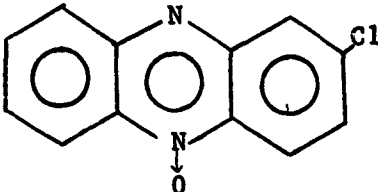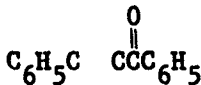
| Compound | Army Primary Screening | | Secondary Screening (Absc./Phyto.) at 5 lb per Acre | | Field Test |
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
(structure) NO_2, Cl, Cl, Cl; $OCNHC_6H_5$	11	20	10/62	-	X
(structure) NO_2, Cl, Cl, Cl; $OCNH$—C$_6$H$_4$Cl	7	18	-	-	-
(structure) NO_2, Cl, Cl, Cl; $OCNHCH_3$	9	20	-	-	X
(structure) NO_2, Cl, Cl, Cl; $OCNHC_4H_9$	15	20	-	19/34	X
(structure) NO_2, Cl, Cl, Cl; $OCNH$—C$_6$H$_3$Cl$_2$	13	19	-	-	-

TABLE 6. RESULTS OF TESTING PHOSPHORUS COMPOUNDS

Compound	Army Primary Screening		Secondary Screening (Absc./Phyto.) at 5 lb per Acre		Field Test
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
$C_6H_4CH_2\overset{O}{\overset{\|}{P}}(OC_4H_2)$	7	17	-	19/43	-
$\underline{p}\text{-}ClC_6H_4CH_2\overset{O}{\overset{\|}{P}}(OC_4H_9)_2$	6	18	-	-	-
$\underline{p}\text{-}CH_3C_6H_4P(NC_5H_{10})_2$	6	17	29/52	12/20	X
$(C_5H_{10}N)_3PO$	7	17	-	6/11	-
$(C_5H_{10}N)_3P$	11	21	-	-	X
$C_5H_{11}\overset{O}{\overset{\|}{P}}(OC_5H_{11})_2$	7	18	-	25/34	-
$C_6H_{11}NH\overset{O}{\overset{\|}{P}}(OC_4H_9)_2$	6	16	-	37/72	-
$(C_4H_9)_2PC\equiv CH$	6	20	-	12/9	-

Table 7 shows four compounds of different types that have shown a good level of defoliant or herbicidal activity. We have here a disulfone, a phenazene, a long chain amine, and an acetylene. We are looking forward to the results of the field tests of these compounds, and we hope in the next two years to prepare more effective defoliants.

TABLE 7. RESULTS OF TESTING MISCELLANEOUS COMPOUNDS

Compound	Army Primary Screening		Secondary Screening (Absc./Phyto.) at 5 lb per Acre		Field Test
	0.1 lb acre	1.0 lb acre	Army	B.T.I.	
$(C_3H_7SO_2CH)_2$	7	16	-	-	-
(phenazine 5-oxide with Cl substituent structure)	10	16	0/29	-	-
$CH_3(CH_2)_{15}N(CH_3)_2$	7	17	-	-	-
$C_6H_5C \overset{O}{\overset{\|}{C}} CC_6H_5$	6	8	-	56/52	X

XIX. MONSANTO GREENHOUSE SCREENING PROGRAM

Philip C. Hamm*

Dr. Koch presented the organizational and functional background for the Monsanto Company as well as the synthesis philosophy or rationale for synthesis modification.

I have been asked to talk briefly about the biological evaluation aspect, i.e., the Greenhouse Screening Program itself.

The particular test plants that we use as yardsticks to measure chemical activity in our screening procedure and the rates of chemical application are as follows: (i) primary screening - Black Valentine bean at 0.1 and 1.0 pound per acre, and the soybean and apple seedling at ten pounds per acre; and (ii) secondary screening - maple, elm, pin oak, privet, euonymus, boxwood, and Ilex all at 5.0, 3.0, 1.0, and 0.25 pound per acre.

The Black Valentine bean and soybean are sprayed after the first trifoliolate leaf has developed and the second one is initiated. The time from seeding to spraying varies from two to three weeks, depending upon weather conditions and the time of year. From mid or late April until mid or late September the time from planting to spraying is two weeks. During the rest of the year the time between planting and spraying is usually three weeks. The difference in age of plants is noted in the record. In general, the contact activity is slightly higher when two-week plants are used than when three-week-old plants are used.

The Black Valentine bean has long been a reference species for detecting the activity of chemicals. It is a good indica or of formative action, desiccant action, defoliation action, necrosis, and kill. Soybean is included in the higher test rate along with the apple seedling to get an indication of the broadleaf activity on an annual species as well as on the woody species. The relatively high rate of ten pounds per acre is used as a screen for possible leads as well as to find active chemicals on both woody and annual species or on either one alone.

In secondary screening except for the first rate of five pounds per acre th order and number of test rates is a function of the activity. Those species that show activity are rechecked at lower rates until the threshold of activity has been established. The normal procedure is to drop to one pound per acre if the material is active at five pounds per acre; then if inactive, the chemical may be checked at three pounds per acre to establish closer limits on the range of activity. Chemicals active as rapid-acting desiccants at five pounds per acre often show

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* Monsanto Company.

defoliant action at three or one pound per acre. The volume of solution used is ten gallons per acre except where solubility causes a formulation problem. In those instances the volume is increased to 20 gallons.

The seven species used in secondary screening (two broadleaf evergreen and five deciduous) were selected for differences in leaf shape, leaf surface, and rate of growth. These differences could modify interception, absorption or penetration and translocation of the chemicals to the active site. In general, it has been observed that more chemicals show defoliant action on deciduous species than on evergreen species.

Table 1 shows the number of quaternary iodides active on more than one tree species at five pounds per acre. Chemicals are grouped according to number of species defoliated 50% within 14 days.

TABLE 1. NUMBER OF QUATERNARY IODIDES CAUSING 50%
OR MORE DEFOLIATION ON TREES
WITHIN 14 DAYS AT FIVE POUNDS PER ACRE

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1 Chemical defoliated 5 species
Maple, elm, pin oak, privet, and euonymus

Of 8 chemicals active on 4 species
8 defoliated privet and euonymus
7 defoliated elm
5 defoliated pin oak
3 defoliated maple
2 defoliated Ilex

Of 21 chemicals active on 3 species
21 defoliated privet and euonymus
18 defoliated elm
2 defoliated pin oak
1 defoliated Ilex

Of 20 chemicals active on 2 species
16 defoliated euonymus
13 defoliated privet
9 defoliated elm
1 defoliated pin oak
1 defoliated maple

Euonymus patens which is considered a semievergreen is defoliated as readily as the deciduous species.

Table 2 shows the number of chemicals that are rapid-acting defoliants, i.e., 50% defoliation within four days or 65% within five days.

TABLE 2.   NUMBER OF QUATERNARY IODIDES
CAUSING 50% OR MORE DEFOLIATION
ON TREES WITHIN FOUR DAYS
AT FIVE POUNDS PER ACRE

Of 3 chemicals active on 2 species
    3 defoliated privet
    2 defoliated elm
    1 defoliated euonymus

Of 14 chemicals active on 1 species
    12 defoliated elm
    2 defoliated privet

The activity of the chemicals in this table is on deciduous and semi-evergreen species. There was no defoliant action on the evergreen species.

Table 3 shows information on the quaternary iodides active at one pound per acre.

TABLE 3.   NUMBER OF QUATERNARY IODIDES
CAUSING 50% OR MORE DEFOLIATION
ON TREES WITHIN 14 DAYS
AT ONE POUND PER ACRE

Of 6 chemicals active on 2 species
    6 defoliated elm and privet

Of 14 chemicals active on 1 species
    11 defoliated elm
    2 defoliated euonymus
    1 defoliated privet

Other iodides active on tree species at five pounds per acre are shown in Table 4.

TABLE 4.   OTHER IODIDES CAUSING 50% OR MORE
DEFOLIATION ON TREES WITHIN 14 DAYS
AT FIVE POUNDS PER ACRE

1 Chemical defoliated 3 species
    Elm, privet, and euonymus

Of 5 chemicals active on 2 species
        4 defoliated privet and euonymus
        1 defoliated elm
        1 defoliated maple

Of 12 chemicals active on 1 species
        7 defoliated euonymus
        3 defoliated privet
        1 defoliated elm
        1 defoliated maple

Table 5 shows another active group of chemicals, the organotin derivatives. The first chemical shown in Table 5 defoliated deciduous, semievergreen, and evergreen species. Its effect on the species treated is unique because of its lack of activity on two deciduous species (pin oak and privet), and its activity on the evergreen species (boxwood and _Ilex_).

TABLE 5.   TIN COMPOUNDS CAUSING 50% OR MORE DEFOLIATION
ON TREES AT FIVE POUNDS PER ACRE

In 14 Days

1 Chemical defoliated 5 species
    Maple, elm, euonymus, boxwood, and <u>Ilex</u>

1 Chemical defoliated 4 species
    Maple, elm, pin oak, and privet

Of 2 chemicals active on 3 species
    2 defoliated privet and euonymus
    1 defoliated <u>Ilex</u>
    1 defoliated maple

In 4 Days

Of 2 chemicals active on 2 species
    2 defoliated euonymus
    1 defoliated <u>Ilex</u>
    1 defoliated privet

1 chemical defoliated privet

XX. EFFECTS OF TORDON ON CROPS

Andrew J. Watson and Keith C. Barrons*

Early in 1963, The Dow Chemical Company released a sample of Tordon —
Dow's trademark for 4-amino-3,5,6-trichloropicolinic acid — to Crops
Division for evaluation. Preliminary screening confirmed its high degree
of activity, particularly on nongrasses. A proposal was submitted by The
Dow Chemical Company to study the effects of this herbicide on ten major
crop species. The work was carried out during the summer of 1963 at Dow's
field stations at Midland, Michigan, and Greenville, Mississippi. Because
dosages used were too high to project accurately the sensitivity of certain
crops, the work was done again at lower dosages during the summer of 1964.
The work will be completed shortly and a final report submitted.

The following crops were used in the experiments: garden bean, soybean,
peanut, potato, sweet potato, sugar beet, wheat, corn, rice, and manioc.

Three-row plots 30-feet long were sprayed with water dispersions at a
volume of 30 gallons per acre. With each crop, Tordon was compared with
the specific herbicide and dosage suggested by Crops Division as being
appropriate on the basis of their earlier research.

The limitations of this technique as a means of accurately projecting
the effect of a compound if applied as a nonaqueous low volume airplane
spray are well-recognized; however, it does serve to indicate relative
activities. Tordon is comparable to 2,4-D and 2,4,5-T in the rate of
foliar absorption and translocation. Thus, it should prove equally
effective in low volume systems.

A. FOLIAR SPRAYS

1. Garden Beans

Garden beans were sprayed at two stages of growth, early bud, and
late fruiting. Tordon was extremely injurious at either stage of growth,
although the earlier treatment was more severe. The lowest rate of Tordon,
0.036 pound per acre, caused yield reductions of 100 and 64% respectively
for the two growth stages. For comparison, 2,4-D amine at 0.25 pound per
acre reduced yield 74% at the early stage of growth; 0.5 pound per acre
caused a 22% reduction at the later stage.

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* The Dow Chemical Company.

2. Potato

Potatoes were sprayed at two stages of growth: (i) when the tubers were beginning to form, and (ii) when the tubers were 1/2 to 3/4 developed. Tordon was extremely active on this crop. With the early treatment, no edible tubers were produced after any Tordon spray (lowest rate used was 0.072 pound per acre) and yield was reduced more than 99% by each of the later treatments. Under these conditions, an early application of 2,4,5-T ester at 0.25 pound per acre caused a 39% yield reduction; 0.5 pound per acre at the later stage reduced yield 55%.

3. Soybean

Soybeans proved very susceptible to Tordon. Treated in midseason, rates as low as 0.036 pound per acre caused 98% reduction in yield. Later treatments were somewhat less injurious, although still highly effective. When sprayed at time of early pod set, rates of 0.036 and 0.072 pound per acre caused a yield reduction of 72 and 94% respectively. 2,4-D Ester at 0.25 pound per acre caused no yield reduction when applied at midseason; 0.5 pound per acre reduced the yield 19% when sprayed at time of early pod set.

4. Peanut

Peanuts were affected seriously by very low rates of Tordon. Yield was reduced 98 to 100% by rates of 0.072 pound per acre or more when the plants were sprayed in early bloom. When sprayed after the seeds were about half developed, yields were reduced 59 and 89% respectively, by dosages of 0.18 and 0.36 pound per acre. 2,4,5-T Ester under these conditions reduced yields 91% at the 0.5 pound per acre rate in early bloom; 1.0 pound per acre caused 63% reduction at the later stage of growth.

5. Sweet Potato

Sweet potato responded readily to Tordon. Rates of 0.072 pound per acre or more reduced yield by 94 to 100% when sprayed as the storage roots were beginning to form. When treatments were applied after the storage roots were three fourths developed, yields were reduced about 80%. For comparison, 2,4-D ester at 0.5 pound per acre reduced yield 94% when applied at the earlier stage of growth; 1.0 pound per acre reduced yield 71% at the later time of treatment.

6. Manioc

Manioc proved far more susceptible to Tordon than was anticipated. When sprayed at the 2.5- to 3-foot stage when storage roots had just started to form, all plants were killed and no edible roots were produced at 0.72 pound per acre, the lowest rate used. This dosage reduced yield about 76%

when spraying was delayed until the plants were seven to eight feet tall and storage roots were about two inches in diameter. Under these conditions, 2,4-D ester at 2.0 pound per acre caused 100% crop destruction when sprayed early; 5.0 pound per acre reduced the yield of edible roots 50% when treated at the later stage of growth.

7. Sugar Beet

Sugar beets were susceptible to Tordon but results were not quite as spectacular as with the other broadleaved crops studied. Sprayed when the roots were about one-half inch in diameter, Tordon reduced root yield 49 and 62% respectively at rates of 0.072 and 0.18 pound per acre. For comparison, 2,4-D caused a 40% reduction at 0.25 pound per acre. Where sprays were delayed until the beets were about four inches in diameter, root yields were reduced only 14% by the highest rate of Tordon, 0.36 pound per acre. Sucrose content of the roots was reduced from 14.5% for the untreated to 6.4% in the 0.36 pound per acre treatment of Tordon applied at the later stage of growth.

8. Wheat

Although spring wheat was not as susceptible to injury as the broadleaved crops studied, it was severely injured by high rates of Tordon when treated in the full tiller or boot stage of growth. A foliage spray at a rate of 0.72 pound per acre caused yield reductions of 81, 93, 8, and 9% respectively when treated in full tiller, boot, early flower, or milk stage of growth. 2,4-D Amine at 0.5 pound per acre reduced yield 11 and 20% respectively at the two earlier stages of growth; 1.0 pound per acre caused 0 and 14% respectively at the two later stages.

9. Corn

Corn was not highly susceptible to Tordon. Injury was greater when sprayed two to three feet tall than when sprayed six to seven feet tall. In the earlier treatment Tordon at the highest rate, 0.72 pound per acre, reduced the yield 57%; only 9% reduction occurred with the later application.

10. Rice

Rice proved relatively tolerant to Tordon. The crop was sprayed at four stages of growth including (i) full tiller, (ii) early boot, (iii) early flowering, and (iv) milk stage. The highest rate of Tordon, 1.4 pound per acre, caused yield reductions of 17, 6, 52, and 32% respectively for the four stages of growth. For comparison, cacodylic acid at 0.5 pound per acre reduced the yield 7 and 97% respectively for the two earlier treatments; 1.0 pound per acre caused 95 and 59% reduction respectively for the two later treatments.

B. SOIL APPLICATIONS

Another phase of the research was to study the residual effect of Tordon in soil on crops planted at different intervals after treating. Tordon at rates of 0.072, 0.36, 0.72, and 1.4 pound per acre and a combination of 2,4-D plus 2,4,5-T each at 1.0 pound per acre were applied to separate areas approximately 9, 6, 3, and 0 (one day) weeks before planting the ten crops used in the foliar spray study. The chemicals were incorporated into the soil with a rotary tiller before planting.

The 2,4-D plus 2,4,5-T treatment caused no symptoms of injury with any of the crops where applied 9, 6, or 3 weeks prior to planting and only moderately severe symptoms occurred on one crop, peanuts, where applied and incorporated one day prior to planting.

Tordon at the two higher dosages caused severe injury to all crops, regardless of waiting period. Most broad-leaved crops were very severely damaged at the lowest rate. There was no indication of chemical loss during the nine-week period. Injury was just as great where the crops were planted nine weeks after application as where planted one day after spraying.

C. CONCLUSIONS

Tordon was highly injurious when applied to nongraminaceous crops. Beans, soybeans, peanut, potato, manioc, and sweet potato were all severely affected at doses far below those required for significant yield reductions from 2,4-D or 2,4,5-T. Sugar beet is affected by Tordon more than by 2,4-D, but the differences are not as great as for the above six crops. The graminaceous crops, wheat, corn, and rice are relatively resistant to Tordon.

The soil residual tests confirmed that Tordon has far greater lasting effects than the halophenoxyacetic acids. Injurious effects are evident for as many as nine weeks after soil applications.

XXI. CONTROL AND DEFOLIATION OF VEGETATION IN AGRICULTURAL RESEARCH SERVICE*

Dayton L. Klingman**

A. INTRODUCTION

1. Personnel

In the 15 months since initiating this research program, all personnel have been recruited and are working in the following areas: (i) two weed scientists and one agricultural engineer in Puerto Rico; (ii) three weed scientists (actually five in closely integrated research) and one agricultural engineer in College Station, Texas; and (iii) two plant taxonomists in Beltsville, Maryland.

2. Facilities

Excellent laboratory and office facilities are furnished under a cooperative program by the Texas Agricultural Experiment Station. They provide 75 acres of irrigated land for a woody plant nursery. In addition, we have built two fiber glass greenhouses and a field laboratory building (temporary) using Advanced Research Projects Agency (ARPA) funds. We have leased field sites in Texas to treat (i) huisache and mesquite at Refugio, (ii) running live oak at Victoria, (iii) mixed hardwoods at Livingston, (iv) whitebrush at Llano, and (v) yaupon, winged elm, and post and blackjack oaks at Carlos and Edge.

In Puerto Rico our personnel are housed at the Federal Experiment Station in Mayaguez. They built a shelter for growing plants for bioassay; also another building was converted to an engineering shop. Areas for research are furnished by the U.S. Forest Service at Luquillo and by the Commonwealth Forestry Department at Maricao and Guanica. Additional areas are leased near Mayaguez.

3. Research

I will start this report by giving a brief summary from our fifth quarterly report of progress; then I will expand on this.

In 1964, between 2,000 and 5,000 plants comprising eight brush species were transplanted in the field nursery in Texas. These plants were to be used for early evaluation of herbicides and for field physiological studies.

In Puerto Rico, special attention is being given to methods of evaluating horizontal and vertical visibility. Some of this research is cooperative with Waterways Experiment Station (WES) and Cold Regions Research and Engineering Laboratory (CRREL).

Further progress was made toward developing an individual tree sprayer to facilitate treating large trees with herbicides in Puerto Rico.

On the basis of information gathered firsthand between November 1963 and January 1964, progress has been made in classifying and establishing the distribution of the principal forest types of Thailand. We continued the identification, classification, and description of Puerto Rican vegetation and related studies.

B. COLLEGE STATION, TEXAS

1. Vegetation Treated in Five Areas in Texas

a. Huisache and Mesquite at Refugio

Herbicide treatments showing most promise from the fall application were used on huisache and mesquite in April 1964. All treatments of paraquat alone and in combination with 5-bromo-3-sec-butyl-6-methyluracil (bromacil), 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 2,4,5-trichloro-phenoxyacetic acid (2,4,5-T), and picloram gave rapid and extensive leaf kill. 2,4,5-Trichlorophenoxyacetic acid gave slower leaf kill than other herbicides.

Many treatments with picloram and dicamba at one pound per acre each resulted in excellent defoliation of huisache within one week. On mesquite, paraquat was more effective in producing leaf kill than picloram, 2,4,5-T, or dicamba at the one and four pound per acre rates.

Evaluation of herbicidal plots nine months after treatments made in October 1963 showed that good control resulted from (i) picloram applied at 1, 2, and 4 pounds per acre, (ii) dicamba at 8 and 12 pounds per acre, and (iii) bromacil at 5 and 10 pounds per acre. All herbicides and herbicide combinations were less effective on mesquite.

b. Running Live Oak at Victoria

Paraquat plus bromacil, paraquat plus 2,4,5-T, paraquat plus picloram, and picloram alone gave most rapid leaf kill in plots treated in April 1964. 2,4,5-Trichlorophenoxyacetic acid gave excellent leaf kill but was slightly slower in response. In May 1964, paraquat, picloram, 2,4,5-T, and dicamba were compared at one and four pounds per acre. Paraquat gave the most rapid and extensive leaf kill the first week after treatment. However, after the first week, leaf kill of live oak was similar for paraquat, picloram, and 2,4,5-T.

Herbicides applied in August and October 1963 were evaluated six months after treatment. Dicamba, 2,4,5-T, picloram, and paraquat showed much promise for control of live oak. Evaluations of these same treatments eight and ten months after application indicated that of all herbicides applied, 5-bromo-3-isopropyl-6-methyluracil (isocil) and bromacil at five and ten pounds per acre gave most persistent control.

c. Mixed Hardwoods at Livingston

Of the treatments applied in the fall, dicamba and the uracils showed the best residual effects but no treatment was completely satisfactory. The most promising treatments were repeated this spring and are being evaluated. Ecological studies have been initiated to determine the type and rate of plant succession on treated plots.

d. Whitebrush at Llano

Evaluations of chemicals were continued at Llano, Texas. In addition to the large plots (22 by 200 feet) treated on May 11, 1964 a number of small plots (20 by 55 feet) were sprayed with a hand boom. These experiments included series with defoliants and desiccants mixed with various additives in an attempt to enhance herbicide translocation. Also, two rates of ten soil sterilants were applied as granules, powders, or liquids to square rod plots on June 9.

e. Post and Blackjack Oaks, Yaupon, and Winged Elm at Carlos and Edge

Among the treatments applied in the summer and fall of 1963, dicamba and bromacil were most effective in preventing regrowth.

In the spring and early summer of 1964, treatments with paraquat were most effective in defoliating yaupon and the oak species. Picloram was most effective on winged elm.

Concurrent with the early spring treatment at Carlos, an evaluation of some of the treatments applied was made in the Woody Plant Nursery at the Texas Agricultural Experiment Station. Early defoliation response was similar at Carlos and at the Nursery.

A study was initiated to get further information on the proper dosage and timing of picloram and 2,4,5-T on yaupon (an evergreen species) and winged elm (a deciduous species). Three rates of each chemical are being evaluated at five dates from early spring through early summer. At equivalent rates picloram defoliated winged elm more rapidly than 2,4,5-T. During the period covered by this experiment, no single optimum date for application appeared to exist.

2. Laboratory Studies

Picloram and maleic hydrazide both strongly inhibit malic dehydrogenase (a component of the Krebs cycle). Kinetic studies of the effect of picloram showed that the inhibition is competitive for nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide reduced (NADH) and noncompetitive for oxalacetate and malate. 2,4-Dichlorophenoxyacetic acid (2,4-D) exhibits a similar inhibition pattern which suggests that the mechanism of action of picloram and the phenoxy herbicides may be the same.

The effect of six herbicide-defoliants on pea glutamine synthetase (an enzyme important in nitrogen metabolism) was studied. Some chemicals did inhibit this enzyme system, but not at herbicidal concentrations.

Studies on the effect of environment on metabolic pathways were begun. Altered metabolic pathways may be the key to defoliant and herbicidal resistance.

Nuclear magnetic reasonance has been used to ascertain chemical structure of uracils.

A number of substituted quinolines have been synthesized and tested for herbicidal activity. Some compounds have shown promise as defoliants.

Conductivity studies with paraquat indicate that membrane damage is enhanced by light.

3. Woody Plant Nursery

The Woody Plant Nursery at College Station, Texas, is expected to be useful for early evaluation of herbicides and for field physiological studies. In 1964, late winter and spring plantings have been completed. Species planted and the numbers of each are as follows: loblolly pine, 2350; yaupon, 3981; live oak, 5266; mesquite, 3946; greenbriar, 5206; whitebrush, 3676; Macartney rose, 3700; and winged elm, 3800.

Survival rates for each species transplanted in June and July 1963 are as follows: 94% for live oak; mesquite, 88%; whitebrush, 61%; winged elm, 59%; and Macartney rose, 50%.

Species transplanted in September and October 1963 have the following establishment rates: greenbriar, 40%; Macartney rose, 85%; and whitebrush, less than 1%. The whitebrush planting was plowed up and replanted in 1964. The Macartney rose and greenbriar plants will be used for late fall 1964 and 1965 studies.

Picloram and 2,4,5-T were applied to mesquite, winged elm, and Macartney rose at 0.25, 1.0, and 4.0 pounds per acre in May and June; 2-methyl-4-chlorophenoxyacetic acid (MCPA) and picloram were applied to

whitebrush at the same rates on the same days. The leaf kill, defoliation, and initial stem kills were similar to those obtained in natural stands of these species. However, plant kills have not been determined.

4. Agricultural Engineering

Preliminary work with Mylar tabs for catching spray samples for evaluating penetration of dense foliage with dyed sprays was started in June. Mylar has been found to be a good adhesive target material by researchers in Oregon. A number of sampling stations were located in brush plots sprayed with oil-in-water and water-in-oil emulsions to determine if small tabs of two sizes would provide an adequate sampling area for studying penetration.

C. PUERTO RICO

1. New Work Initiated

a. Work on Bamboo

Arrangements have been made with Eureka Central to use bamboo (Bambusa vulgaris) on their land for evaluating herbicides. A few preliminary foliage applications have been made with herbicides known to be toxic to grasses. The bulk of the treatments will be made as soon as possible.

b. Vertical Visibility

Vertical visibility is now being measured from the ground with a camera with a 180 degree angle of acceptance. Canopy photos have been taken of 62 plots on the island. Additional canopy photos will be taken of the same plots at two-month intervals. The first photos are now being developed, so an analysis has not been made.

The amount of clear sky will first be estimated and then accurately determined by planimetry. It is hoped that visual estimates will be accurate enough to negate the need for time-consuming planimetry. Determination of vertical visibility from the ground will be correlated with measurements of optical density and vertical visibility from the air by aerial photography.

c. Horizontal Visibility

Cooperative work has been started with the San Juan Office of the Waterways Experiment Station (WES), U.S. Army Corps of Engineers. WES is also concerned with visibility but they are approaching the problem differently. In the cooperative endeavor WES is using Agricultural Research Service (ARS) plots to test their techniques in assessing visibility. WES is testing horizontal visibility by taking stereo pairs of photographs along

four radii from the centers of plots. They are attempting to assess verti-
cal visibility by measuring optical density (OD). OD is defined as the
$\log_{10}$ of the ratio of light above the forest canopy to that on the ground
below the canopy. OD should be high before treatment of the vegetation
with herbicides and decrease as defoliation progresses. OD measurements
will be related to the amount of visible sky as determined by planimetry
of canopy photos.

d. Aerial Photography

Cooperative work is also underway with the U.S. Army Cold
Region Research and Engineering Laboratory (CRREL). One of our problems
has been to find equipment for aerial photography. It was learned throug:,
WES that CRREL sends a professional photographer to Puerto Rico every two
months on an aerial photo mission. Aircraft for those missions are
arranged by WES from Fort Brooke. We have arranged with CRREL to have
their photographer take pictures of our sites. CRREL will have the use
of the negatives for photo interpretation work and we get the needed
aerial photos. A detailed explanation of the objectives of the aerial
photography is discussed in another section (Section C, 4).

2. Soil-Applied Herbicides

The effect, movement, and persistence of herbicides in three dif-
ferent soils and rainfall zones were studied on tropical arborescent
species. The objectives, procedures, and preliminary results are dis-
cussed in the 1963 Annual Report, Defoliation Project, Mayaguez, Puerto
Rico.

Results to date from each of the three test areas have been sum-
marized and are reported herein. Each location is discussed separately
as to defoliation. Soil residues are discussed in general terms for all
locations.

Positive identification has not been completed for all species in
each location; however, the total number of species in the sampling area
of each location is: Guanica, 28; Maricao, 129; and Luquillo, 93.

Drypetes glauca is represented at both Maricao and Luquillo as
one of the eight principal species in each location. It is probably a
heterogeneous taxon. Differentiation of sterile specimens from Casearia
is difficult if not impossible.

a. Desiccation and Defoliation

(1) Guanica

Defoliation data were collected 6 and 7 May 1964. Check plots were defoliated 50% because of the deciduous nature of some species during the dry season. Accumulated rainfall was 9.63 inches from the date of herbicide application to June 29, 1964.

All herbicide treatments except dicamba at 9 and 27 pounds per acre, and 2,3,6-trichlorophenylacetic acid (fenac) at 9 pounds per acre resulted in increased defoliation as compared to data collected in January 1964 (1963 Annual Report). However, some of the increased defoliation might have been due to the extended dry weather. Picloram gave the highest over-all defoliation followed by bromacil. The decreased effectiveness of dicamba may have been partially due to its apparent disappearance from the soil. In general, 2-methoxy-4,6-bis (isopropylamino)-s-triazine (prometone), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), and fenac have been relatively ineffective as soil-applied treatments.

(2) Maricao

Picloram was the most effective soil-applied herbicide followed by dicamba, bromacil, and fenac. From a practical viewpoint, picloram was the only herbicide that gave good defoliation. At 9 and 27 pounds per acre it appeared to effectively desiccate and defoliate Alsophila aquilina, Casearia decandra, Miconia sintenisii, and Drypetes glauca. Dicamba defoliated Rapanea ferfuginea, C. decandra, and M. sintenisii at 27 pounds per acre. All other herbicide treatments were relatively ineffective on individual species and as broad-spectrum defoliants. Accumulated rainfall from herbicide application date to June 29, 1964 has been 33.63 inches.

(3) Luquilla

Data collected four months after the application of soil treatments indicated that picloram and dicamba were the most effective herbicides in desiccating and defoliating the principal species present. Bromacil would be ranked third in over-all activity but at this date the activity of bromacil, prometone, diuron, and fenac is too low to be of importance.

Some differential species susceptibility was present. Tabebuia heterophylla and Cordia borinquensis were somewhat resistant to picloram. C. borinquensis was also somewhat resistant to dicamba. Euterpe montana, Drypetes glauca, Psychotria beteriana, and Inga fagifolia were very susceptible to picloram at 27 pounds per acre. P. beteriana and I. fagifolia gave some indications of extreme susceptibility to dicamba at 9 pounds per acre. The variability between herbicide rates cannot be explained at this time.

Accumulated rainfall from date of herbicide application to May 18, 1964 has been 18.33 inches.

b. Soil Residues

All herbicides at all rates at all locations moved downward in the soil to a depth of 48 inches within three months after application. Picloram, dicamba, and fenac apparently moved downward in the soils more rapidly than bromacil and prometone. Diuron appeared to move down very slowly. This was indicated by rather low toxicity to cucumber indicator plants in soil samples collected at depths below 24 inches three months after application. The higher the initial rate of herbicide application, the greater the concentration of herbicide throughout the soil profile to a depth of 48 inches.

Dicamba produced less total toxicity to cucumber indicator plants in soil samples collected three months after application than the other herbicides. Data on soil samples collected at Guanica six months after application indicated most of the dicamba had disappeared. These preliminary results indicate dicamba has much less soil persistence than the other herbicides used in this study.

c. Summary of Soil Treatments

Data collected six weeks after treatment at Maricao and Luquillo did not indicate significant desiccation or defoliation.

Data taken five to seven months after herbicides were applied to soil indicated that picloram, dicamba, and bromacil were effective in defoliating some species of woody plants in Puerto Rico. However, this defoliation is not rapid and possibly would be useful only in combination with more rapid-acting herbicides. These data also indicate that rates of 9 pounds per acre or more are needed to defoliate plants with the individual herbicides used in this study.

An individual herbicide did not defoliate a broad spectrum of plants when applied to the soil. This suggests that a combination of herbicides should be applied as a single treatment if the defoliation of many species is of primary concern.

It is not known at the present time whether species that have been completely defoliated are dead. Data taken during the next six months will tentatively confirm whether or not death has occurred.

Residue studies have shown that the herbicides used in this study moved down through the soil profile to a depth of 48 inches within three months of application. Water-soluble herbicides (picloram, dicamba, and fenac) appeared to move more rapidly than herbicides that are less

water-soluble. There is an indication that dicamba will not persist for extremely long periods in the soil. Samples collected from Guanica Commonwealth Forest six months after application indicated almost complete disappearance of dicamba.

3. Susceptibility Studies

a. Seasonal

In tests of seasonal susceptibility propyleneglycolbutylether esters of 2,4-D and 2,4,5-T at six pound acid equivalent in 100 gallons (aehg) in a water carrier gave neither desiccation nor defoliation as early as one week after treatment. The two-week and one-month observations indicate increased effectiveness during the middle portion of the testing period. The increased effectiveness may be associated with a period of low rainfall. Although a rain gauge at the site was not in operation during that period, recorded rainfall at the FES, only a few miles away, was only 3.53 inches for the four-month period of December through March. Average rainfall for the period is almost ten inches. Work must progress over a much longer period before conclusions can be drawn.

b. Herbicide Treatments during 1963 and 1964

The objective of this phase is to evaluate a wide range of herbicides for their effectiveness on guava. Treatments were made in October and December 1963 and in March and June 1964.

(1) October 1963

Wetting sprays were applied in mid-October with a compressed-air sprayer at six pound aehg in a water carrier.

Observations at six months show 2,4-D, dicamba, and picloram to be the most effective herbicides; bromacil was quite effective at 13 pounds active ingredient per 100 gallons (aihg). A mixture of 2,4-D and 2,4,5-T was not as effective as 2,4-D or 2,4,5-T alone.

(2) December 1963

A mixture of 3-amino-1,2,4-triazole-ammonium thiocyanate (Amitrole-T) and diuron was omitted in this test because it showed so little promise in the first test. The defoliants that caused rapid but short time defoliation were tested again with a surfactant to determine if defoliation could be maintained for a longer period of time. Only data from six months' observation are discussed.

Picloram and dicamba were clearly the most effective herbicides. The addition of X-77 to dicamba was, as expected, beneficial.

(3) March 1964

Additional herbicides were eliminated for the March treatments because they lacked promise. Those herbicides used were tested more intensively for the effect of surfactants.

An antifoam agent added to picloram accelerated the rate of response; however, at the end of one month the effect of the antifoam agent was no longer apparent.

c. Coffee

Coffee (Coffea arabica) was treated in March 1964 because it was considered important to learn about the susceptibility of commercially important crop plants as well as the undesirable woody plants.

Diquat and paraquat caused very rapid desiccation. Defoliation at the end of a month was almost as high as that from the more effective systemic herbicides. Further passage of time will show the duration of defoliation.

4. Measurement of Vertical Visibility by Aerial Photography

As mentioned in Section C, 1, d, the aerial photography is being done through cooperation with CRREL. Aerial photographs of treated plots will be taken every two months. As a measure of visibility, white sheets of masonite (2 by 8 feet) are placed at eight-foot intervals along the center line of the long axis of each plot of one replication at each of three sites - Guanica, Maricao, and Luquillo.

Four types of film were used on the first photo mission flown on May 27, 1964; panchromatic, color, infrared, and camouflage detection. From these films we will select two that best suit our needs. Future photos will be taken with the two emulsions selected. Negatives from the May 27 photo mission have not been received.

5. Summary of Visibility Studies

Four techniques are being tested in vertical visibility studies.

a. Vertical Photos from Ground

Vertical photographs from the ground are taken with a canopy camera (180 degree angle of acceptance). These photos are viewed as being the most exact representation of degree of visibility obscuration because the amount of sky visible from a given point can be closely determined

water-soluble. There is an indication that dicamba will not persist for extremely long periods in the soil. Samples collected from Guanica Commonwealth Forest six months after application indicated almost complete disappearance of dicamba.

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by planimetry. Consequently, the canopy photos will be used as the standard against which other systems are compared.

b. Optical Density Measurements

Optical density will be measured by personnel of WES. OD $= \log_{10} \frac{T}{G}$, where T = radiation above the forest canopy and G = radiation near the forest floor. As vegetation density increases, progressively more light is intercepted; so G decreases and consequently OD increases. OD thus has an inverse relationship to vegetation density.

The sensing units of the WES equipment have a sensitivity range similar to that of the visible spectrum. They are an improvement over previous sensors that accepted too much radiation in the IR region.

Optical density measurements will be taken at five-foot intervals along eight radii from the plot centers for a total of 61 measurements per plot. When the data are collected, regression analyses will be made using "area of visible sky" from the canopy photos as the independent variable and different densities of OD measurements as the dependent variables. If the variation is not too great, we should then be able to express OD in terms of visibility - something that is not now possible. The hope for OD is that the number of measurements can be reduced to the point where the system would be much faster than canopy photography.

c. Aerial Photography of Visibility Targets on Ground

Five targets (two by eight feet) are placed on the center line of the long axis of each plot. Air-to-ground visibility will be expressed as the number of targets that can be seen on the photograph.

The data collected as described in the preceding paragraph will also be subjected to regression analysis using amount of visible sky from the canopy photos as the independent variable.

d. Light Transmittance Measurements from Aerial Photographs

Negatives of aerial photographs will be analyzed optically with a microdensitometer to determine possible differences in light transmittance attributed to different vegetation densities. If the data appear promising, they will also be subjected to regression analysis using amount of visible sky as the independent variable.

From the four methods described above, we hope to develop a rapid but simple and accurate method of assessing visibility. It may also be possible to predict horizontal visibility if vertical visibility is known, but the possibility is admittedly very tenuous at present.

6. Engineering Work

The engineering phases of the project have been hampered by rough, mountainous terrain of the location available for the work and the lag time in obtaining materials from the States.

a. Cableway

The design of the cableway to be used for studying spray distribution and spray penetration of a dense forest canopy was completed. It calls for two 120-foot towers and two 35-foot towers. The heights of the towers were dictated by the irregular slope of the site and the height of the top cover of the vegetation. The location of the site makes it almost mandatory that these towers be set by helicopter, which, in turn, imposes a maximum weight limit for the towers. The local power company developed a method of using helicopters to set poles or towers and has a trained crew to work with the helicopter pilot. In discussions with company engineers, they pointed out the weight limit on towers set by helicopter and suggested the possibility of using aluminum alloy towers similar to those they had obtained from the Michael Flynn Manufacturing Company that are 90 feet high and are capable of supporting a single cable under 12,000 pounds' tension. These towers are freestanding ar⁰ are supported on a ball and socket joint at the bottom and by four g, wires running from the top of the tower to anchors set 90 degrees ap, around the tower.

The cableway site has been laid out, and the clearing of trees and brush at the tower and anchor locations is under way.

The site for an automatic weather station has been located, and a small metal shed has been set up to house the recording gear. The components for the station have been obtained and assembly is nearly completed. This station will be a semipermanent installation at the cableway site. However, it is designed for portability and can be moved to other locations.

b. Equipment for Spraying Individual Trees

A pneumatically operated mast suitable to support a spray boom has been designed and ordered. This mast will support a 20-foot spray boom for spraying individual trees. The spray delivery from the boom will be graduated to provide equal coverage on the area included in a circle ranging in diameter from 20 feet or less to 40 feet. A broad jet nozzle will deliver the spray beyond the boom.

D. BELTSVILLE, MARYLAND

   1. Vegetation of Southeast Asia

       Progress has been made during the past quarter in classifying and
establishing the distribution of the principal forest types of Thailand
on the basis of information gathered firsthand during November 1963 and
January 1964.

       This information is collated with data extrapolated from reports
published by Maurand, Rollet, and other foresters and ecologists prior to
the dissolution of French Indochina.

       A comparison has been made between the forests of Southeast Asia
and Puerto Rico from studies of April 1963. Although the components of
the forests in these widely separated areas may belong to entirely different
ent species, genera, and even families, they exhibit similarity of
physiognomy and other analogous characters, as well as types such as
mangrove forests.

       A comprehensive report is now nearing completion discussing the
criteria to be considered in classifying tropical forests, primarily of
Southeast Asia.

       Further information is still required on gallery or riparian
forests flanking rivers and streams, especially in eastern and north-
eastern Thailand; this information is vital from a military standpoint.

       Another problem that requires further studies in Southeast Asia
is plant succession, particularly in climax rain and moist evergreen
forests. We are familiar with the dominant species of fast-growing trees,
shrubs (Broussonetia and Trema), and grasses such as "lalang" (Imperata),
Saccharum spontaneum and Neyraudia sp. that appear along trails and road-
sides, or bamboos that soon invade clearings of teak and deciduous forests,
but studies on plant succession in the dense, humid forests in Southeast
Thailand and in the Southern Peninsula still remain to be made.

       The task of reviewing and annotating published reports on the
vegetation of the countries of Southeast Asia under consideration is
continuing.

       The data now assembled will be the basis for publishing a series
of illustrated articles on the forests and forest products of Thailand
and Southeast Asia in general.

2. Vegetation of Puerto Rico

April was spent in the field in Puerto Rico gathering data pursuant to the following objectives: (i) studying sunlight incidence on the forest floor, (ii) collecting and studying tropical tree seeds and seedlings, (iii) classifying and describing forest types in Puerto Rico, (iv) identifying some of the remaining problematical species in the defoliation plots, and (v) compiling phenological data on tropical trees.

During May and June, seeds collected in Puerto Rico were planted and the resultant seedlings studied and illustrated. Probing experiments have been initiated to see how herbicides will affect the morphology of the seedlings. So far, more than 25 species of seedlings have been studied. Other seeds were purchased from seed companies specializing in tropical seeds to expand coverage. Furthermore, a survey of the rather limited literature on tropical tree seedlings is well under way. Pertinent data are being entered in an information retrieval system. These seedling studies will unquestionably facilitate studies on secondary succession after defoliation in the tropics.

Literature work has continued in several directions: (i) updating nomenclature of Britton and Wilson's Flora of Puerto Rico; (ii) combining three approaches to forest classification, i.e. floristic, physiognomic, and holistic; and (iii) searching for survival and hazard plants of Puerto Rico.

A finalized listing of the species encountered in the herbicide plots is near completion.

E. CONCLUSION

I have tried to briefly review work underway. We are confident that herbicides of greater activity will be found as a result of the concentrated effort being made by commercial companies and partially supported under contract. Evaluation of such new herbicides is necessary and time-consuming. We believe the research program that we have under way and that of Fort Detrick and others that involve plant families provide information with good potential for extrapolating to many other situations. Well-trained, intelligent, and energetic weed scientists are engaged in these research programs. I believe we cannot fail to make good progress if time is allowed to reap the benefits from the research.

Locust was the most susceptible of all. Death occurred from Tordon, 2,4-D, "Orange," Tordon plus 2,4-D, Tordon plus "Orange," Tordon plus endothal, and Tordon plus diquat. When diquat alone was used, there was almost complete recovery in approximately three weeks, and now the tree shows no effect from the spray.

B. 1963 FIELD TRIALS AT FORT MEADE

At Fort Meade last year 24 plots, each 225 square feet, were sprayed with cacodylic acid, Dowco 173, and butynediol at 10, 25, 40, 55, 70, 85, and 100 pounds per acre on 15 species of trees. Cacodylic acid was the most effective compound used in these tests. At ten pounds per acre, it killed all vegetation in the plot and at 40 pounds per acre it caused defoliation of pine. On re-examining these plots this spring, all plots sprayed with cacodylic acid appeared dead, but regrowth was evident on the plots sprayed with other compounds.

The species sprayed were: scrub pine, red maple, pin oak, willow oak, common persimmon, quaking aspen, black cherry, sweet gum, black locust, American chestnut, huckleberry, greenbrier, blackberry, sumac, and viburnum.

C. 1964 FIELD TRIALS AT FORT MEADE

This year at Fort Meade we sprayed 105 plots, each 225 square feet, with 52 different compounds at five- and ten-pound rates. This area contained 43 different tree species with approximately four species per plot. Some plots were on abandoned farm land where apple, pear, and lilac were growing, species not native in forest areas. Trees, particularly those growing in direct sunlight, are browning out from the compounds sprayed and are showing some leaf drop. Included in the 52 compounds are 23 compounds obtained by contracts under synthesis. This is the first time in the two years of this program that we have had contract compounds active enough to be tried on small plots.

D. 1964 FIELD TRIALS IN GEORGIA AND TENNESSEE

Crops Division arranged with Georgia Power Company and Tennessee Valley Authority for the use of 65 acres of right-of-way through the swamps of Georgia and an additional 65 acres of right-of-way in the mountains of Tennessee. These acres were sprayed with selected compounds with their helicopters and using their personnel. Compounds used in both areas were commercial herbicides because they were available in quantity and also because commercial helicopters can be insured against crop damage only when the compounds used meet approval of USDA.

In Georgia "Orange," "Purple," diquat plus Tordon, Tordon, dicamba, and diquat were used on several species of oak, three species of pine, pond cypress, chinaberry, river birch, wild cherry, hawthorn, sweetgum, red maple, sumac, ash, willow, cottonwood, palmetto, and bay. One plot in particular was covered with fast growing species including maple and pond cypress. This area had been cut two years ago but some pond cypress were 18 feet tall at the time of spraying. We sprayed this area with a combination of diquat and Tordon. Wilting was evident on maple in one-half hour, and brownout was obvious within ten days after spraying. If the vegetation on this plot reacts similarly to that on other plots when sprayed with diquat plus Tordon, we should get almost complete kill in this area.

In order o test a different range of species under different climatic conditions with the same compounds used in Georgia, we asked TVA for a mountainous region. This area is located 90 miles northeast of Chattanooga and is mostly in the Cherokee National Forest. The species here consist of white pine, short-leaf pine, scrub pine, rhododendron, kalmia, sourwood, oaks, hickory, hemlock, ash, alder, sweetgum, maple, and dogwood.

We used four of the same compounds that we used in Georgia but substituted butynediol, Butyrac 2 (2,4-DB), and Veon BK (amines of 2,4-D and 2,4,5-T) in place of diquat, endothal, and cacodylic acid because TVA corrosion tests showed that these compounds were too corrosive to spray on their towers and insulators. These three compounds were sprayed on a right-of-way outside of Chattanooga with a ground rig.

The plots in Tennessee were observed the third week of July. Tordon was very effective on pines and several other species, but it was not effective on ash or rhododendron. Butynediol caused tip burn on the leaves of most species, but no permanent effects. Dicamba is slow-acting, so the effect at this time was not too noticeable. Tordon plus Veon was no more effective than the Tordon 101 formulation that was used. "Orange" was applied in a stand of almost all hardwoods that browned out quickly. "Purple" was applied in a transitional area of pines mixed with hardwoods. It browned the hardwoods quickly but did not have much effect on the pines.

Final evaluations will be made next year on the 1964 treatments.

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13 ABSTRACT

The research program in chemical defoliation of the U.S. Army Biological Laboratories and its affiliated contractors and agencies is summarized in this Proceedings of the 1964 Defoliation Conference.

Four phases of the effort are reviewed: (i) basic studies in the mechanisms of leaf abscission by contract and in-house research; (ii) synthesis of new chemicals as candidate defoliants, desiccants, or herbicides; (iii) screening and evaluation of candidate chemicals by in-house and contract programs; and (iv) field testing of promising defoliants under in-house and USDA programs.

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